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Phenotyping Outpatient Exacerbations in Bronchiectasis.

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This thesis is dedicated to my mother Ranjit Sidhu for her unfailing belief in me in all aspects of my life and who I hope would have been proud. Also to my husband Timothy, eldest daughter Olivia and baby girl Victoria for giving me the motivation to complete this degree.

CONTENTS

DECLARATION

This thesis describes work undertaken in the University of Edinburgh's Centre for Inflammation Research, Queen's Medical Research Institute, Centre for Infectious Diseases and the Department of Respiratory Medicine, Royal Infirmary of Edinburgh from August 2013 to July 2015. The work described in this thesis has been my own and the writing of this thesis has been entirely my own undertaking. The validation of the incremental shuttle walk test has been accepted for publication in *CHEST* and other relevant publications arising from this thesis constitute the appendix. This work has not previously been submitted for a higher degree or other professional qualification.

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ABSTRACT

Bronchiectasis is a chronic respiratory condition with a wide spectrum of disease severity. There is a pressing need for randomised controlled trials in bronchiectasis to help guide management of this condition which is associated with increased morbidity and mortality. There is paucity of data in this respiratory condition but it is a rapidly progressive field. Most studies are conducted in more severe disease (colonised with *Pseudomonas aeruginosa*, 3 or more exacerbations/year, reduced lung function) as they experience more exacerbations which in turn reduces quality of life and increases mortality. However, the vicious cycle of bronchiectasis implies this is a progressive disease whereby infection leads to a destructive inflammatory response which destroys the mucociliary escalator which in turn leads to a build-up of stagnant mucus and further perpetuates the cycle by predisposing to further infection. Treatment strategies are being aimed at breaking different part of this vicious cycle and should aim to prevent exacerbations. A logical starting point for treatment could be in those with less severe disease and thereby hope to prevent progression to more severe disease. Yet, little is known about outpatient exacerbations which could be classed as less severe exacerbations of bronchiectasis.

The aim of this thesis was to phenotype outpatient bronchiectasis exacerbations. In particular, the aim was to investigate the importance of bacterial load in these exacerbations to see whether they could have influence the diagnosis and management of outpatient exacerbations. There are few clinical endpoints in bronchiectasis that have been validated for use in bronchiectasis that could assess treatment response. Further endpoints are urgently needed to help assess randomised controlled trials which could investigate novel treatment options to guide evidence based management plans. This thesis also aimed to assess the incremental shuttle walk test, a marker of functional capacity, as a validated clinical endpoint for use in bronchiectasis.

This thesis reported several clinical markers that could correlate with the Bronchiectasis Severity Index in a cohort of 207 patients with bronchiectasis. Sputum colour, 24 hr sputum volume, incremental shuttle walk test, parameters of

lung function, quality of life assessments, serum inflammatory markers and sputum inflammatory markers all correlated with Bronchiectasis Severity Index tertiles of increasing disease severity. Further work is necessary in a larger cohort to assess whether these clinical endpoints could be additive to the BSI in predicting hospital admission and 30-day mortality rates.

This thesis examined 94 exacerbations of bronchiectasis that were all managed as an outpatient with 14 days of oral antibiotics based on previous sputum microbiology results. All clinical parameters assessed (sputum colour, spontaneous sputum volume, 24-hour sputum volume, the Leicester Cough Questionnaire score, the St George's Respiratory Questionnaire score, FEV_1 and FVC actual and predicted scores, serum inflammatory markers; white cell count, neutrophil count, erythrocyte sedimentation rate and C-reactive protein, sputum inflammatory markers myeloperoxidase and trends in neutrophil elastase) all deteriorated from baseline to start of exacerbation. Bacteriology was investigated and demonstrated an increase in culture positive sputum samples for pathogenic bacteria with a change in dominant pathogen in over 53% of samples. There was a 2-log increase in bacterial load CFU/ml from baseline to start of exacerbation overall but when sub-analysed, this was only present when the dominant pathogen changed, i.e. there was no increase in bacterial load if the dominant pathogen stayed the same. This is the first study to demonstrate a change in dominant pathogen leads to an increase in bacterial load. The clinical significance of a 1log or more rise in bacterial load was explored and found to be associated with increased severity markers of 10% or more decline in actual and predicted FEV_1, 5% or more reduction in incremental shuttle walk test score, increase in 24hour sputum volume, increase in sputum inflammatory markers myeloperoxidase and neutrophil elastase, doubling of white cell count and 50% increase in neutrophil count at start of exacerbation. A rise of 1 or more log unit in bacterial count CFU/ml was also associated with a symptom complex of chest pain, increased sputum volume and the absence of headache. This symptom complex is more specific for a rise in bacterial load than either the British Thoracic Society or European consensus definition of an exacerbation.

Lastly, this thesis assessed the incremental shuttle walk test as a validated clinical endpoint for use in patients with bronchiectasis. Its reliability, validity and responsiveness were demonstrated with no change in incremental shuttle walk test over 6 months of clinical stability, correlation with other validated endpoints (including the St George's Respiratory Questionnaire (SGRQ) total and activity score, the MRC dyspnoea score, the Bronchiectasis Severity Index score and physical activity duration and sedentary time recorded on activity monitors) and improvement with different antibiotic therapies for exacerbations and stable bronchiectasis with intravenous, oral and nebulised antibiotics. The minimum clinically important difference was found to a 5% improvement in walk distance as this took patient's variable baseline functional capacity into account as well as correlating well with patients who had a clinically important improvement in SGRQ score (4 or more unit improvement).

This thesis provides interesting results which require further investigation to help manage patients with bronchiectasis in the outpatient setting. Larger randomised controlled trials are needed to assess the clinical importance of a rise in bacterial load and to differentiate at which threshold colonisation becomes infection and requires treatment. This could ultimately lead onto randomised controlled trials that manage exacerbations based on bacterial load and symptoms, such as is currently the case in treating urinary tract infections. Once a rise in bacterial load has been further clarified, it could also provide insight into the duration of antibiotics needed in a cohort of patients that rely heavily on antibiotic therapy and antibiotic stewardship in a climate where there is a finite amount of available treatment options. Further external validation of the incremental shuttle walk test would strengthen its recommendation for use in routine clinical practice and research studies to assess response to existing and new therapies.

LAY SUMMARY

Bronchiectasis is a progressive condition that affects the lungs and can range from mild to severe. Bronchiectasis causes patients to have a troublesome cough and produce thick sputum on a regular basis because the airways in the lungs are abnormally widened. There is a pressing need for research studies in bronchiectasis to help provide evidence based medicine to patients of this condition which is associated with increased mortality.

Most studies are conducted in more severe disease as these patients are more affected daily with a poorer quality of life as they are prone to more frequent infections. However, bronchiectasis is a disease that can get worse with each infection as infections are thought to cause local damage to the airways, thereby studies should be directed at investigating less severe disease to prevent infections and the disease from getting worse. Little is known about infections of bronchiectasis in patients that can be treated in the community i.e. have less severe infections than those managed in the hospital.

The aim of this thesis was to investigate patients with bronchiectasis that are managed in the community when they are well and when they are suffering from an infection related to their bronchiectasis. A further aim was to investigate if the number of bugs present in the sputum of patients during an infection was important. There are few assessments in bronchiectasis that have been studied that can reliably assess whether patients have responded to treatment. It is important to be able to determine if new therapies are helpful and so more assessments that can assess therapies are needed. This thesis also aimed to assess if the incremental shuttle walk test; a walking test that assesses a patient's exercise capacity, can be used to accurately measure a patient's response to treatment in bronchiectasis.

This thesis reported several different tests that are not currently included in the Bronchiectasis Severity Index (BSI) – a scoring system that assesses disease severity, could associate with the BSI scores in a group of 207 patients with bronchiectasis. Sputum colour, 24 hr sputum volume, incremental shuttle walk test,

lung function, assessment of a patient's quality of life and markers found in the blood and sputum all correlated with the BSI, i.e. there were worse test results for each assessment with higher (worse) BSI scores. Further work is necessary in a larger group of patients to investigate whether these additional tests could improve the BSI scoring system in predicting hospital admission and rates of death.

This thesis examined 94 patients with bronchiectasis that suffered from a chest infection who were all managed out of hospital with a 14-day course of tablet antibiotics. All tests performed (sputum colour, volume of sputum collected the day before and in the morning of the visit, the Leicester Cough Questionnaire score, the St George's Respiratory Questionnaire score, lung function tests, blood tests and sputum tests had all deteriorated during an infection compared to when the patient had performed them when they were well. Often the type of bug found in the sputum was different to what had previously been present in the patient's sputum and it is thought that this change had led to a large increase in the number of overall bugs present in the sputum during an infection. This is important because the larger the number of bugs present, the worse the patient's condition is likely to be.

Lastly, this thesis assessed the incremental shuttle walk test. It was shown to be reliable; not change with time, valid; as good as other tests that assess exercise and responsive; test scores improved when patients were given treatments that we know improve their health (antibiotics). A 5% change in the test score was found to be the threshold at which point a patient is said to have experienced a change in condition.

This thesis provides interesting results in patients with bronchiectasis that are managed out of hospital. Larger studies are needed to further investigate what an increase in sputum bugs means for the patient. Once this has been established, the way in which we diagnose infections and when and for how long we treat them may change. There is a limited number of different antibiotics available so it is important to only prescribe them when necessary. The incremental shuttle walk test needs to be tested in other centres to further strengthen its ability to assess response to new and existing treatments in bronchiectasis.

ABBREVIATIONS

BSI	=	Bronchiectasis Severity Index
CFU	=	Colony forming units
COPD	=	Chronic obstructive pulmonary disease
CRP	=	C-reactive protein
ESR	=	Erythrocyte sedimentation rate
FEF_{25-75}	=	Forced expiratory flow at 25-75%
FEV_1	=	Forced expiratory volume in 1 second
FVC	=	Forced vital capacity
HRCT	=	High resolution computer tomography
IL-8	=	Interleukin-8
ISWT	=	Incremental shuttle walk test
LCQ	=	Leicester Cough Questionnaire
MPO	=	Myeloperoxidase
MRC	=	Medical Research Council
NE	=	Neutrophil elastase
SGRQ	=	St George's Respiratory Questionnaire
WCC	=	White cell count
VO	=	Baseline visit
VS	=	Start of exacerbation visit
VE	=	End of exacerbation visit

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Chapter 1:
Introduction

1.0 Introduction

1.1 Bronchiectasis

Bronchiectasis is a term derived from the Greek words '*bronkhia*' denoting the branches of the main bronchi and '*ektasis*' meaning dilatation. The term 'bronchiectasis' describes the condition it was named after in the most simplistic terms and with updates in modern day knowledge there are now clinical and radiological subdivisions within this condition.

1.2 Background

Bronchiectasis was first described by the French physician Rene Laennec, in 1819. He writes a chapter entitled 'De la Dilatation des Bronches' in his famous book 'De l' Auscultation Mediate ou Traite des maladies des poumons et du Coeur' (Laennec, 1819). He described how bronchioles can dilate to the width of a 'crow's feather, a goose quill, or even a finger' and how these can abruptly regain their normal width or terminate in a 'tortuous cul-de-sac' or present as cavities capable of 'accommodating a hemp seed, a cherry pit, an almond kernel or even a walnut' (Laennec, 1834 Lindskog, 1986). He also described how this dilatation of bronchioles would become permanent, in patients that were afflicted with 'chronic mucous catarrh', because of bulky sputum mass blocking the airways.

This condition was further described by Sir William Osler in the late 1800s who is believed to have died from the complications of bronchiectasis himself but it wasn't until 1922 that further detailed anatomical knowledge about bronchiectasis was discovered. Sicard and Forestier visualised the bronchial tree by injecting radio-opaque materials like iodized oil alongside radiographic imaging – a procedure later known as bronchography (Sicard 1922, Joress 1944). This technique allowed the condition to be diagnosed at a much earlier stage and the presence, exact location and extent of disease could be detected. Thereafter, the most comprehensive descriptions were elucidated by Reid in 1950 when she examined the bronchograms and lung resections of 45 patients with a primary diagnosis of bronchiectasis. Reid classified the cases in three groups based on the predominate type of bronchial dilatation. Group I – 'cylindrical' type of bronchiectasis described by a uniform

appearance with slight dilatation with an abrupt ending, Group II – 'varicose' bronchiectasis is irregular forms of dilatation with sites of relative constriction and Group III – 'saccular' or cystic bronchiectasis described as balloon-like dilatation increasing towards the periphery of the lung – see Figure 1 (Reid, 1950).

Figure 1.

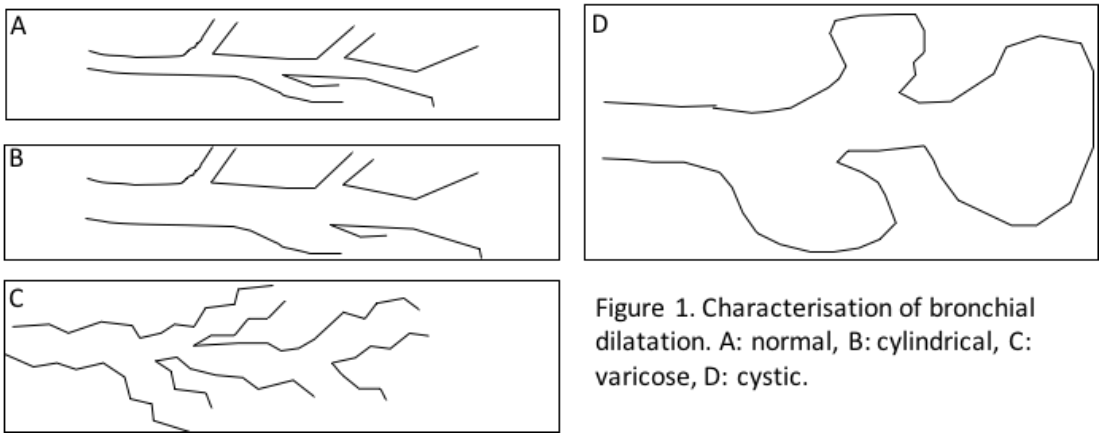


Figure 1. Characterisation of bronchial dilatation. A: normal, B: cylindrical, C: varicose, D: cystic.

1.3 Pathophysiology

The pathogenesis of bronchiectasis is poorly understood. Bronchiectasis is the permanent dilatation of airways in the context of chronic infection and inflammation (Barker 2002). The exact mechanism is not known but elastin in the airways is thought to be lost and in more advanced disease this is proceeded by loss of muscle and cartilage (Whitwell 1952). A predominantly neutrophilic inflammatory response in the airway lumen ensues (Ella 1994) but despite this immune response over two thirds of patients remain colonised with potentially pathogenic micro-organisms (Angrill 2002, Chalmers 2012). The driver for the persistent neutrophilic response is not known but infection is thought to play a role (Angrill 2001). It has been postulated that the increase in neutrophils is due to a failure of resolution of inflammation in addition to a persistent infective driver. Activated neutrophils produce neutrophil elastase, a protease that breaks down elastin, as well as other proteins like tumour necrosis factor - alpha (TNF-α), interleukin-8 (IL-8) and myeloperoxidase that are found to be high in airway inflammation (Angrill 2001). These proteins correlate with bacterial load (Chalmers 2012) and they together with bacterial products can cause structural damage and dilation of small airways.

1.3.1 Mucociliary clearance structure

The mucociliary clearance system is part of the innate host defence system responsible for maintaining lung sterility. The passage of air from the pharynx to the alveoli where gas exchange occurs is via the respiratory tract. This tract is lined with a respiratory epithelium which serves to moisten and protect the airways. It comprises of columnar epithelial cells, goblet cells and basal cells. The columnar cells are ciliated, and function to move overlying mucus towards the oropharynx. The goblet cells contain membrane-bound mucous granules and secrete mucus to make up the epithelial lining fluid that maintains moisture and traps any airway pathogens or particulate matter. The basal cells can respond to areas of injury and differentiate into other cell types to maintain an intact epithelial lining (Figure 2).

Figure 2.

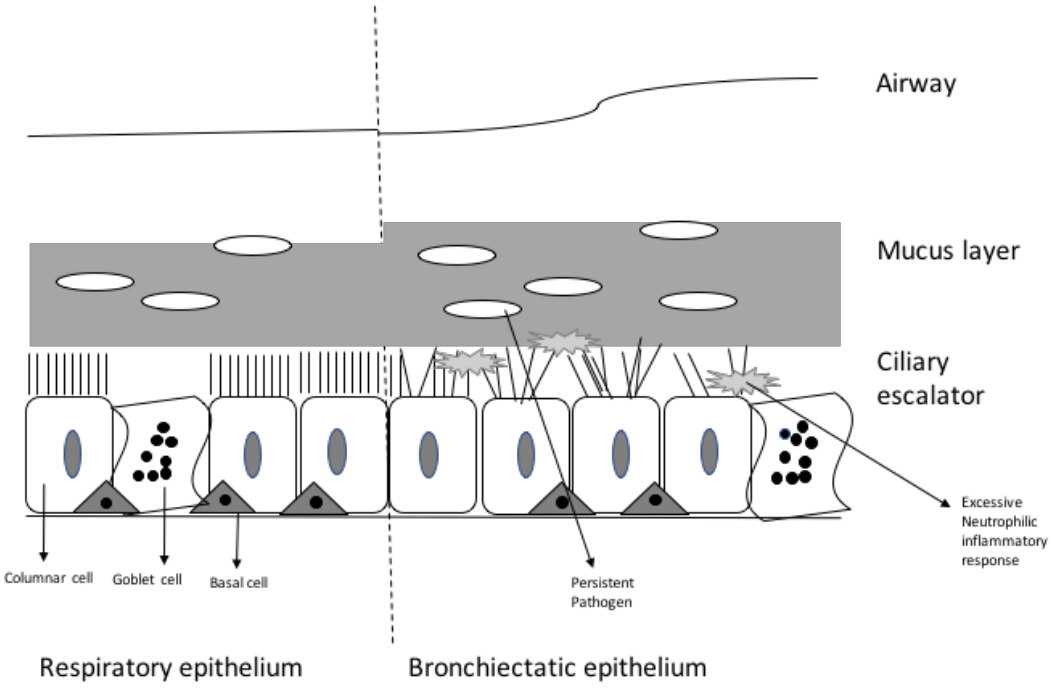


Figure 2. Left: normal epithelial lining, right: abnormal mucociliary escalator due to neutrophilic inflammatory response with mucus build up and dilated airway seen in bronchiectasis.

The ciliated columnar epithelial cells are present in the respiratory tract as far distally at the 17th airway division. Each cell has about 200 cilia present on its

surface which are approximately 6μm long and 0.25μm in diameter (Greenstone & Cole 1985). In the respiratory tract, the cilia beat in a rhythmical continuous coordinated fashion in a two-layer system of fluid (Lucas & Douglas, 1934). The extent of the cilia lie in a watery solution called the periciliary fluid just beneath the thicker layer of mucus. To effectively move mucus along the tract with its associated trapped particles, the cilia perform two movements. The cilia have hooks on their tips which engage with the under surface of the mucus layer (Jeffrey & Reid 1977). They then propel the mucus forward in the intended direction of travel by standing erect above the cell surface. After the 'effective' stroke the cilia return to their starting point by sweeping at right angles across the cell surface within the periciliary fluid termed the 'recovery' beat, so as not to engage the above mucus and cancel out the initial effective stroke.

The secretory cells release mucins, defensins and lysozomes (antimicrobial agents), cytokines (immunomodulators) and protective molecules which integrate with water to form the thick mucus layer (epithelial lining fluid). This thick gel layer consists of 97% water and 3% mucins. It functions to trap airway matter to expel it from the lungs. The airway mucus accumulates as it is transported through the larger airways and eventually it reaches the trachea and then the pharynx where it is unnoticeably swallowed. If, however there is a build-up of mucus then a cough is triggered to augment expectoration.

The efficiency and integrity of the mucociliary escalator is essential in maintaining airway sterility. A deficient mucus layer or poor ciliary structure or function will allow a build-up of mucus that is exposed to the airways for longer. The airways will then be exposed to the pathogens or toxins held by the mucus and this will trigger a predominantly neutrophilic inflammatory response. In bronchiectasis, the airways are permanently dilated and distorted with a resultant impaired mucociliary escalator (Figure 2).

Each cilium consists of a central pair of filaments surrounded by nine additional pairs of filaments (A and B microtubules) in a circumferential design. This circular

border is known as the axoneme and is surrounded by an extension of the cell membrane. The microfilaments join proximally to terminate in a basal body from which root fibres extend into the cell cytoplasm. Arising from midway up the basal body is the 'basal foot' and this points in the direction of travel of the effective stroke of the cilia, presumably to act as anchorage for the motile cilium. Each pair of outer microtubules has projections called nexin links to adjacent microfilaments and radial spokes attaching them to the inner central microfilament (Figure 3). The outer microfilament pairs slide over adjacent pairs by using energy gained from hydrolysing adenine triphosphate (ATP) in its outer projection dynein arms. The radial spokes limit movement but also transduce this into coordinated activity (Greenstone & Cole 1985).

Figure 3.

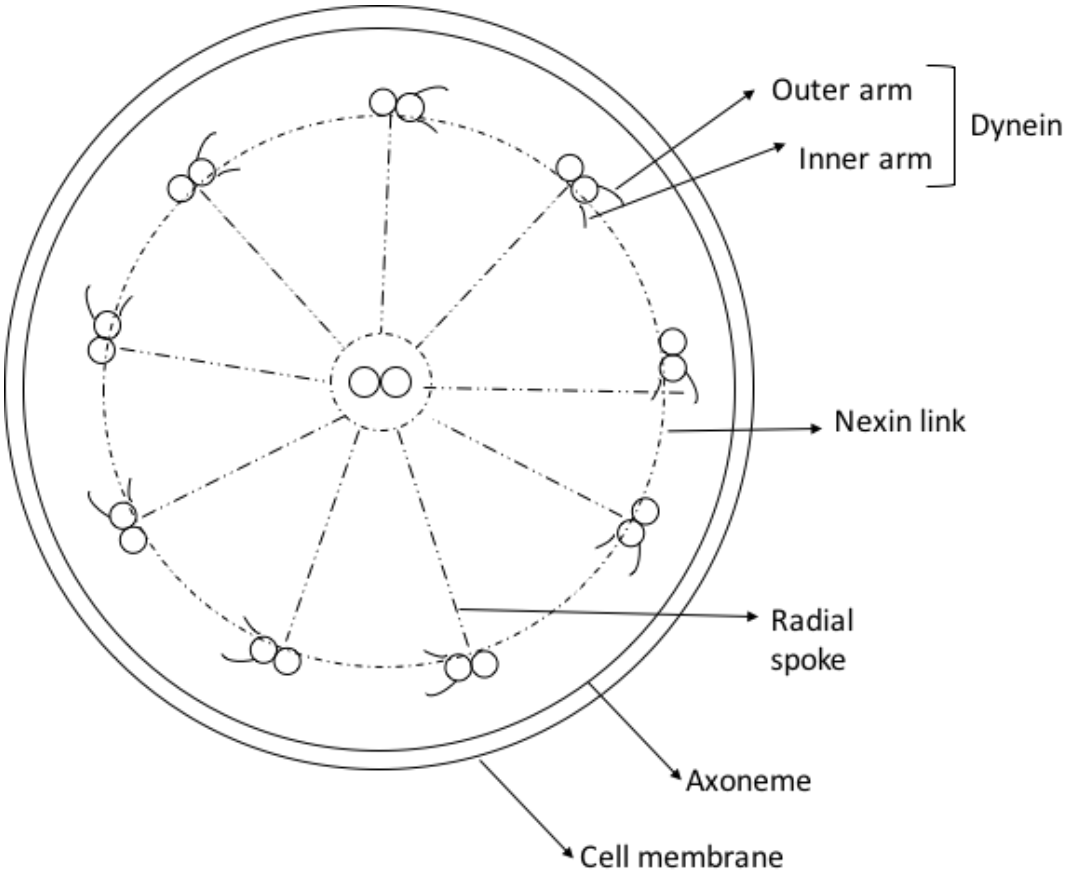


Figure 3. Classical structure of normal cilium (Adapted from Buchdahl *et al,* 1988).

1.3.2 Abnormal ciliary structure

The precise structure of the cilium is integral to maintaining its function. There are limited studies of ciliary abnormalities in bronchiectasis and are mainly limited to patients diagnosed with primary ciliary dyskinesia – an autosomal recessive condition that causes ultrastructural abnormalities of the cilia. The recognised abnormalities include the loss of the "9+2" formation, abnormalities in the orientation of the central microtubules, deficiencies or absence of the dynein arms and abnormalities of the nexin links between the pairs of peripheral microtubules (Sturgess *et al*, 1979 & Cowan *et al*, 2001).

Tsang *et al* investigated the angle of central microfilament orientation in 133 patients with bronchiectasis and in 59 control patients. 103 of these patients had idiopathic bronchiectasis and 6 had Kartagener's. The central microfilament orientation angle correlates with reduced ciliary beat frequency and the presence of structural defects. They found a significant correlation with lower central microtubular orientation angle in patients with severe bronchiectasis (Forced expiratory volume in 1 second (FEV_1) <60%, Forced vital capacity (FVC) <60% or more than 4 bronchiectatic lung lobes) $p<0.05$ (Tsang *et al*, 2005c). A second study by the same group in 152 stable patients with idiopathic bronchiectasis demonstrated slower ciliary beat frequency ($p<0.05$) and greater percentage of patients with central and peripheral microtubular defects than in control subjects (OR 14.4, 95% CI 5.6-36.8). There was no clinical correlation with a slower ciliary beat frequency but the percent of cilia with central microtubular defects correlated with 24hour sputum volume production ($r=0.40$, $p=0.001$) and FEV_1 ($r=.-0.24$, $p=0.01$) suggesting a pathogenic role for central microtubular defects in idiopathic bronchiectasis (Tsang *et al*, 2005b).

1.3.3 Abnormal ciliary function

To move mucus effectively across the mucociliary escalator, cilia must beat at a normal rate, in a constant direction and in a co-ordinated fashion (Eliezer *et al*, 1970). It is thought that cilia beat more slowly in bronchiectasis and it is due to the presence of purulent secretions (Rutland & Cole, 1981, Tsang *et al*, 1995b & Wilson

et al, 1986). Several factors affect ciliary function such as excessive release of inflammatory markers like elastase (Tegner *et al*, 1979) and exposure to toxic bacterial products (Wilson *et al*, 1985).

1.4 Vicious Circle

The airways of the respiratory tract are lined with a moist respiratory epithelium. This bears cilia – 'hair like' structures surrounded by mucus or epithelial lining fluid. Mucus traps foreign matter and pathogens in the airways and travels via the coordinated beating of the cilia up the airways towards the pharynx (Stanke 2015, Sleigh 1988). Here it is silently swallowed or coughed up to aid the expulsion of such sepsis-inducing material from the lower airways. The rhythmic co-ordinated action of the cilia action is referred to as the mucociliary escalator and in bronchiectasis where the airways are dilated and distorted this mucociliary escalator can break down. This leads to the build-up of mucus, which when lying stagnant can become prone to infection. Such infections, again cause an inflammatory response and again leads to further dilation and distortion of the airways. This theory is known as 'the vicious circle' and was initially coined by Cole *et al* and is demonstrated in figure 4 (Cole 1989).

Figure 4.

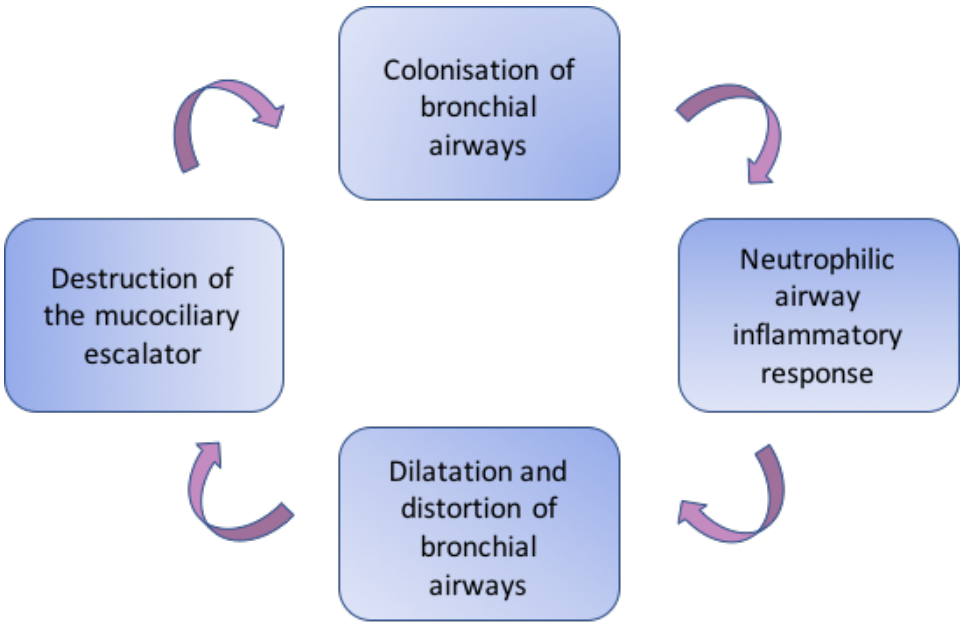


Figure 4. Schematic of Cole's Vicious Circle.

1.5 Aetiology

Bronchiectasis is a pathological endpoint of irreversibly damaged and dilated airways which can be associated with multiple different disease processes. All the different aetiologies can eventually result in the damage of the mucociliary escalator on the respiratory epithelium, build-up of mucus with colonisation and infection and subsequent inflammation. This further perpetuates the cycle causing dilation and distortion of the airways. Therefore, there is emphasis on identifying the primary insult to treat the underlying condition wherever possible.

Investigating the aetiology is important in avoiding invasive, time-consuming and expensive tests in addition to guiding management. Pasteur *et al* investigated 150 adult cases of bronchiectasis and found the underlying aetiology in 47% of cases. In 15% of these cases the causative factor had important therapeutic and prognostic implications, such as allergic bronchopulmonary aspergillosis (ABPA) requiring treatment with steroids and common variable immune deficiency (CVID) with intravenous immunoglobulin therapy (Pasteur 2000). This rate was found to be higher at 56% in 136 cases of paediatric bronchiectasis (Li 2005).

The rate of 'idiopathic' or no cause identified and different aetiological diagnoses can vary greatly between studies and this is probably due to different research techniques. The population of patients included can differ in age, socioeconomic background, background disease incidences, referral from secondary or tertiary centres and the range of investigations performed can vary greatly (Pasteur 2010). Table 1 shows the typical incidence of the different aetiologies and the supporting diagnostic features (Pasteur *et al*, 2010 & Pasteur *et al*, 2000).

Table 1.

Aetiology	Incidence	Supporting features
Idiopathic	41-53%	• Exclusion of other diagnoses
Post infection	29-33%	• History of previous infection e.g. pneumonia, whooping cough, measles, tuberculosis • CT changes of previous infection e.g. tuberculosis
Immunodeficiency (agammaglobulinaemia, CVID, IgA deficiency, IgG subclass deficiency)	1-8%	• Low levels of either IgA, IgM, IgG or IgG subclasses (1-4) • Poor antibody response against Tetanus toxoid and the polysaccharide capsules of *Streptococcus pneumoniae* and *Haemophilus influenzae type B* • Recurrent mucosal infections with encapsulated organisms • Recurrent upper and lower respiratory tract infections with *Streptococcus pneumoniae*, *Haemophilus influenzae* & *Moraxella catarrhalis*
Allergic bronchopulmonary aspergillosis	1-7%	• History of asthma • Raised total and specific IgE to *Aspergillus* • Fleeting infiltrates on chest x-ray and CT • Proximal bronchiectasis on CT • Peripheral blood eosinophilia • Raised Aspergillus precipitins
Connective tissue	3-6%	• History and clinical examination

disease		characteristic of syndromes • Autoimmune antibody and anti-CCP screen
Aspiration or inhalation	4%	• History consistent with aspiration • Positive findings at bronchoscopy e.g. foreign body or mucus plugging • Localised bronchiectasis
Cystic fibrosis	3%	• Age less than 40yrs old • History of malabsorption, steatorrhoea, diabetes and male infertility • Persistent isolation of *Staphylococcus aureus* in sputum • Positive for cystic fibrosis transmembrane regulator receptor TR mutations • Positive sweat test
Ciliary dysfunction	2%	• History of chronic otitis media, continuous rhinitis, infertility and dextrocardia • Exhaled nasal nitric oxide test • Abnormal ciliary beat pattern and frequency on brushings from two separate sites • Genetic conditions
Inflammatory bowel disease	1%	• History of malabsorption, weight loss, joint pains, diarrhoea

		• Diagnostic colonic biopsy
Congenital	<1%	• History of congenital defects of large airways, tracheobronchial or oesophagobronchial fistula, rib malformations or tracheobronchomegaly

Table 1. Aetiologies of bronchiectasis with incidences and supporting diagnostic features. CT: computer tomography, CFTR: cystic fibrosis transmembrane regulator receptor.

Previous infections suffered are a common precipitant for adult bronchiectasis such as bacterial pneumonia, whooping cough, mycoplasma and tuberculosis. Viral causes of bronchiectasis that cause permanent lung damage and subsequent bronchiectasis include *adenovirus*, *measles virus, influenzae* and *respiratory syncytial virus*. The closer the link of pulmonary infection to the development of chronic respiratory symptoms, the more likely the infection was the cause of the bronchiectasis. Studies suggest that patients are relatively good at identifying a significant respiratory infection that could be responsible and hence taking a detailed history is important in helping to limit unnecessary investigations (Nicotra *et al*, 1995 & Wynn-Williams 1953). Sometimes however, an infection can proceed chronic symptoms by years and whilst this can make identifying the aetiology difficult, it is still possible (Lanning *et al*, 1980).

Mycobacterium tuberculosis infection can precipitate bronchiectasis, often in a lobar or segmental distribution (Parker *et al* 1968), and the incidence depends on the population prevalence of the infection. Other mycobacterial species (non-tuberculous mycobacteria, NTM) are ubiquitous environmental organisms and the environment has been proposed as the major source of NTM infection. The most common pathogens include *Mycobacterium avium complex* (MAC), *Mycobacterium abscessus*, and *Mycobacterium chelonae*. Opportunistic environmental mycobacteria can cause bronchial wall damage and hence bronchiectasis but patients with damaged lungs (including those already diagnosed with bronchiectasis) are also at a

higher risk of contracting the infection with these opportunistic organisms in the first place. Patients with bronchiectasis are predisposed to NTM infection in either a localised or widespread distribution and can be isolated in sputum of up to 10% of bronchiectatic patients (Fowler *et al* 2006). Levin and colleagues have reported the presence of bronchiectasis and parenchymal nodules had an overall sensitivity of 80% and specificity of 87% for positive MAC culture (Levin 2002). A meta-analysis by Chu and colleagues after searching the main databases including PubMed, Medline, Cochrane Library and EMBASE identified 8 studies reporting on NTM prevalence in 1492 patients with bronchiectasis (Chu *et al*, 2014). Using the random effect model they found the combined prevalence of NTM in the meta-analysis cohort to be 9.3%. They also reported increased combined NTM prevalence in those studies with 100 or more subjects, studies performed in 2002 and after, in Asian countries and in studies conducted retrospectively.

The clinical significance of isolating mycobacteria in sputum is not known and these patients should be monitored and treatment considered when colonisation with 2 positive sputum samples appears alongside a clinical (reduced lung function and infective symptoms) and radiological deterioration (rapid progression of disease, cavitating nodules, mucus plugging and tree in bud bronchiolitis) in the patient's wellbeing. At this point you may well consider the picture has progressed from colonisation to infection. The different type of mycobacterial species (*mycobacterial avium complex, mycobacterium kansasii, mycobacterium malmoense*) may also influence the clinical picture and when to consider treatment (Pasteur *et al* 2010). For example, *Mycobacterium avium complex*, which is associated with infection of the middle lobe or lingular in women - termed 'The Lady Windermere syndrome', may develop bronchiectasis over several years (Reich & Johnson 1992).

Primary defects in lung defences causing bronchiectasis in more commonly diagnosed in children unless the defect is subtle and not identified until adulthood. The more common defects include primary ciliary dyskinesia (PCD) and common variable immune deficiency (CVID) where antibody production and/or function is defective. The immune defects only constitute a small number of cases and as such,

the exact immune defect tends to be poorly characterised. It is important to attempt to clarify the immune defect as administration of intravenous immunoglobulin G (IgG) therapy in CVID can help prevent infections and pneumonia but there is no consensus on the dose of IgG to administer or what trough levels to aim. There is no evidence that treatment prevents the progression of bronchiectasis. Additionally, in patients with immune deficiency the baseline specific antibody levels against capsular polysaccharides of *S. pneumoniae* should be measured. If found to be low, then 23 valent pneumococcal vaccine should be offered followed by rechecking antibodies levels 4-8weeks after. If patients do not mount an appropriate serological response then the 13-valent pneumococcal vaccine should be considered (Hill *et al*, 2018). Primary ciliary dyskinesia is an autosomal recessive condition with structurally abnormal cilia. It is characterised by upper and lower respiratory tract infections, chronic sinopulmonary infections, bronchiectasis and loss of lung function. Male infertility and situs inversus (Kartagener's syndrome) is also common (Hill *et al,* 2018). Diagnosis is made by measuring nasal nitric oxide in the first instance but analysing nasal brushings for ciliary beat frequency and pattern by either transmission electron microscopy or high speed video microscopy analysis, have high sensitivity and specificity also (Hill *et al,* 2018).

Allergic bronchopulmonary aspergillosis (ABPA) is an important cause of bronchiectasis and should be identified because treatment can halt progression of lung damage. It can be diagnosed with deterioration on symptoms (exacerbations, increased sputum production, cough, wheeze and reducing lung function), serological testing (high total and specific IgE to *Aspergillus fumigatus*), immediate cutaneous reaction to Aspergillus (positive skin prick test >3mm) and new radiological changes (new pulmonary infiltrates and/or high attenuation mucus plugging, in the later stages, proximal bronchiectasis develops). ABPA is very often associated with asthma and it can be difficult to distinguish between the two as the above blood tests can be raised in both conditions, although usually to a lesser extent in asthma. Another diagnostic challenge with ABPA is that the bronchiectasis changes may be identified when the condition is quiescent and blood tests near the normal range. High resolution CT scan should help elucidate the cause as ABPA

causes predominantly central bronchiectasis in the upper lobes but can effect all lobes and in a peripheral distribution (Angus *et al*, 1994 & Mitchell *et al* 2000).

Raised IgG to *A. fumigatus,* raised aspergillus precipitins (which can also be raised in other conditions such as aspergilloma and chronic pulmonary aspergillosis) and raised peripheral blood eosinophilia (which can be raised but normal levels do not rule out ABPA as there is no difference in counts between patients with aspergillus sensitisation and those with ABPA) should all be second line confirmatory tests (Hill *et al,* 2018).

Aspiration of a foreign object can cause bronchiectasis because the secretions produced distal to the obstruction build up and can cause inflammation and structural wall damage. In the same manner, endobronchial tumours - benign or malignant can similarly cause distal bronchiectasis (Box *et al*, 1991 & Chiu 1973). There is benefit to removing the obstruction wherever possible with some improvement of the bronchiectasis (Mansour *et al*, 1998).

Aspiration of gastrointestinal contents has been documented to cause bronchiectasis as has gastro-oesophageal reflux disease. There has been conflicting evidenced as to whether infection with Helicobacter pylori is causative (Tsang *et al* 1998b, Angrill *et al* 2006 & Gulhan *et al* 2007) but heart and lung transplant patients with documented oesophageal dysmotility and reflux were found to have bronchiectasis in a high frequency (Reid *et al*, 1990).

Bronchiectasis is associated with many systemic disease entities. The most common being rheumatoid arthritis which is a chronic inflammatory disease -primarily affecting the joints but with extra-articular manifestations affecting the eyes, skin, heart and lungs. Walker described an association with bronchiectasis in 3.1% of 516 patients with rheumatoid arthritis (Walker, 1967). A further study which investigated 77 patients with HRCT showed the most common pulmonary abnormality to be bronchiectasis in 30% of patients. Of these, 8% did not have any respiratory symptoms (Cortet *et al*, 1995). Whilst an association is well recognised,

the pathogenesis is not understood and there is no consensus on the prognosis of this specific subset of patients (Swinson *et al* 1997, McMahon *et al* 1993). De Soyza and colleagues recently investigated 147 patients with bronchiectasis and rheumatoid arthritis overlap syndrome (BROS) and found a higher mortality rate over a mean of 48months when compared with idiopathic bronchiectasis (18% versus 9.3%), statistically but not clinically significant increase in bronchiectasis severity index scores (7.7 versus 7.1) and no difference in hospitalisation or exacerbations (De Soyza *et al*, 2017). Systemic sclerosis, ankylosing spondylitis, systemic lupus erythematosus, relapsing polychrondritis, Marfan's syndrome and Ehlers Danlos' syndrome are all conditions that have been known to have patients with bronchiectasis in their cohorts but studies are too few and too small for an association to be properly investigated (Pasteur *et al*, 2010).

Cystic fibrosis (CF) is an important consideration when diagnosing bronchiectasis as both conditions can present with recurrent lower respiratory tract infections. Late first presentation of CF as an adult has been described and atypical presentations with respiratory symptoms in the absence of pancreatic failure or gastrointestinal symptoms has been known. A sweat chloride test and genetic testing for homozygosity of the CF transmembrane regulator mutation or heterozygosity for other mutations should be conducted (Pasteur *et al*, 2010).

Obstructive airways disease is often seen in patients with bronchiectasis. Airway changes such as airway thickening and dilatation are seen on high resolution CT (HRCT) scan in patients with asthma. Features of bronchiectasis can also be seen with a higher frequency than in control subjects (Grenier *et al*, 1996). Bronchiectasis is strongly associated with patients with severe asthma and fixed airway obstruction and one study of asthmatic patients that excluded those with ABPA found varicose bronchiectasis in 60% of severe non-allergic type patients and cylindrical bronchiectasis in 50% of severe allergic type patients (Paganin *et al*, 1996). There have been no studies to date to investigate if asthma, independent of ABPA, is a cause of bronchiectasis.

Bronchiectasis has been found in patients with Chronic Obstructive Pulmonary Disease (COPD) (Patel *et al*, 2004, O'Brien *et al*, 2000) but documentation about smoking history and other causes of bronchiectasis has never been investigated thoroughly enough to establish whether COPD is causative of bronchiectasis or not. One study of patients with COPD demonstrated the isolation of *Pseudomonas aeruginosa* in sputum culture of patients with COPD are more likely to have bronchiectasis and that bronchiectasis was an independent risk factor for all-cause mortality in COPD (Mao *et al*, 2015). A meta-analysis of 6 studies in COPD patients reported a 54.3% prevalence of bronchiectasis comorbidity (range 25.6-69%), patients were more likely to have exacerbations, worse lung function, increased colonisation with *Pseudomonas aeruginosa* and other potentially pathogenic microorganisms and a higher smoking history (Ni *et al*, 2015). A second meta-analysis of 14 studies in COPD patients reported patients with bronchiectasis comorbidity were more likely to have worse airway obstruction, increased colonisation with potentially pathogenic micro-organisms, increased exacerbations and mortality rates (Du *et al,* 2016).

1.6 Clinical features

Patients with bronchiectasis suffer from many different symptoms but the most commonly reported is cough. More than 90% of patient report cough and this can be productive of sputum on a daily basis for more than 75% of patients (Pasteur *et al*, 2010). Approximately 5-8% of patients have a dry cough and the remaining patients produce sputum intermittently (Pasteur *et al*, 2010). Cough has been known to reduce a person's quality of life as some find it irritating and others embarrassing. Thus, a specific questionnaire – The Leicester Cough Questionnaire has been validated to assess quality of life in patients with bronchiectasis who suffer from a cough (Murray *et al,* 2009).

Sputum production can be assessed for volume and degree of purulence. Sputum should be collected over a 24hour period and measured or weighed. The amount of sputum produced can vary greatly among patients with some patient producing none and others producing copious amounts when clinically stable. Sputum production

can increase with infections but some patients find they are unable to expectorate when unwell. Sputum colour should also be assessed in patients with bronchiectasis. Increased sputum purulence is caused by neutrophilic inflammation with increased levels of myeloperoxidase and neutrophil elastase (Stockley *et al*, 2001). A study of 141 patients' sputum categorised sputum into mucoid, mucopurulent and purulent. They found increasing purulence correlated with colonisation and severity of radiological changes. Those with cystic or varicose bronchiectasis, as opposed to tubular bronchiectasis, tended to produce more purulent sputum (Murray *et al*, 2009).

Dyspnoea is a common symptom, present in three quarters of all cases. The degree of breathlessness correlates to the degree of FEV_1 obstruction. Breathlessness is also found to correlate with the amount of sputum produced and radiological changes on HRCT (Smith *et al*, 1996 & Ellis *et al*, 1981).

Haemoptysis can sometimes be an alarming clinical feature for patients but often is minor and accompanied by an infective exacerbation. It is present to some degree in approximately 50% of cases (Nicotra *et al*, 1995 & Warner 1935) but only 4% experience massive haemoptysis (>235mls) (Wynn-Williams 1953). Haemoptysis usually arises from aberrant blood vessels from bronchial arteries and should be monitored incase embolisation of the bleeding vessel is required.

Chest pain is reported between 19% and 46.3% of patients with bronchiectasis (Nicotra *et al*, 1995 & King *et al*, 2006) can be pleuritic or musculoskeletal in nature. It can be poorly localised but one study found that 94.4% of chest pains reported in patients with bronchiectasis were described to be in the same area as the bronchiectatic lung (Munro *et al*, 1989).

1.6.1 Clinical signs

The most common clinical sign is audible coarse crackles on auscultation of the chest. Crackles tend to be typically heard at the lung bases but can be heard anywhere. There is poor correlation of the localisation of crackles with the

bronchiectatic lung underneath (Smith *et al*, 1996). Crackles are present in approximately 70% of cases and start in early inspiration. Wheeze has been reported in 34%, large airway rhonchi in 44% and finger clubbing can occur but this is an infrequent finding (Nicotra *et al*, 1995 & Wynn-williams *et al* 1953). Often the clinical examination can be unremarkable.

1.6.2 Stable state

Bronchiectasis is a chronic condition and patients will suffer periods of stability and periods of deterioration. A patient with bronchiectasis may well be symptomatic of the condition on a day-to-day basis experiencing the above named symptoms. They will, however, feel well in themselves and can function at their usual level of ability. Sputum from patients when they are 'stable' may well culture potentially pathogenic microorganisms as their lungs become colonised with bacteria due to the nature of the condition. They may not require antibiotics at this stage but should continue to perform chest physiotherapy regularly to prevent infections.

There have been some studies which suggest there is ongoing chronic infection in patient during the stable state and hence the ongoing neutrophilic inflammatory response. Chalmers and colleagues have demonstrated that airway bacterial load correlates with airway inflammatory markers (Chalmers *et al*, 2012) and hence there has been further study into the effects of anti-inflammatory therapies in patients that are clinically stable to prevent further exacerbations (Wong *et al*, 2012, Altenburg *et al*, 2013, Serisier *et al*, 2013). Tunney and colleagues investigated bacterial load and taxonomy in patients that were clinically stable (no antibiotics for 4 weeks prior to recruitment). They found the bacterial load was similar during the stable state with that in patients who were experiencing an exacerbation. They also found multiple DNA sequences (15,000) that represented codes for 113 distinct microbial taxa, all of which were present in low abundance (Tunney *et al*, 2015). The latter studies are conflicting to the data found using the culture-based methodologies. The 16S studies however were carried out in a small number of bronchiectasis patients and larger studies are needed.

1.6.3 Exacerbations

The British Thoracic Society (BTS) guidelines state an exacerbation is when a patient experiences an acute deterioration in their symptoms over the course of several days. They may experience more cough, increased sputum volume, increased sputum viscosity, an increase in the sputum purulence with or without increased breathlessness, wheeze, haemoptysis and/or systemic upset (figure 5). The BTS guidelines suggest prompt antibiotic treatment at times of an exacerbation to break the perpetual cycle of bronchiectasis and prevent further damage to the airways. Studies have shown that exacerbations impact on a patient's quality of life and the higher the number of exacerbations, the poorer the quality of life (Wilson *et al*, 1997). The BTS audit conducted nationally reports the number of exacerbations can vary anywhere from 0-12, depending on the severity of the bronchiectasis but the average number of bronchiectatic exacerbations of patients managed in secondary care is 2 (Hill *et al*, 2012).

Figure 5.

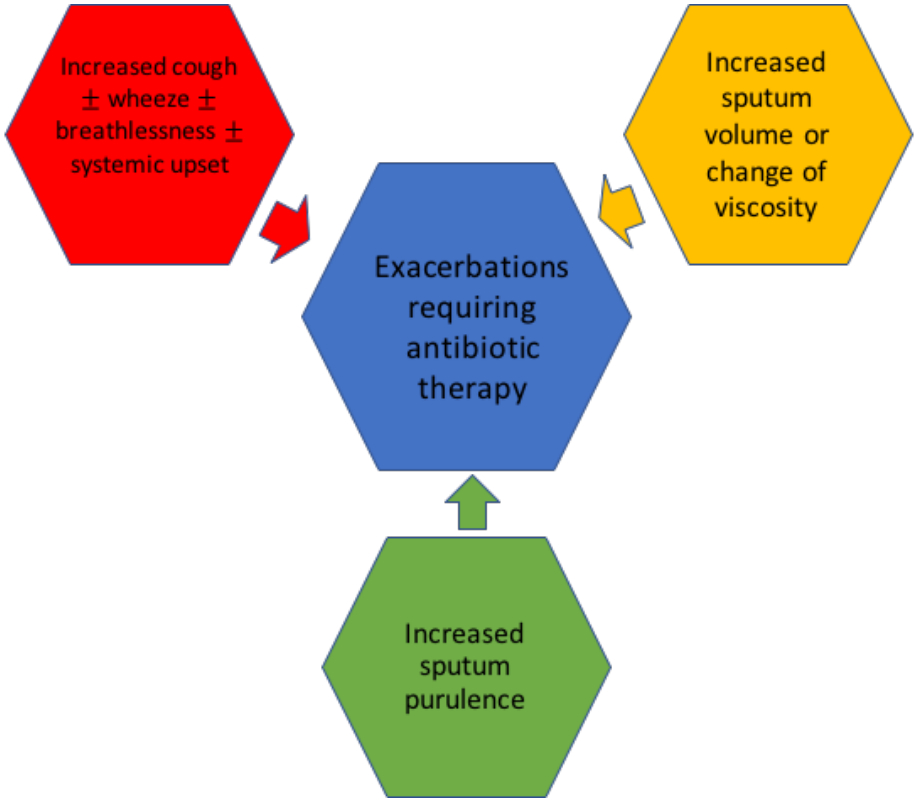


Figure 5. Exacerbations as defined by the British Thoracic guidelines (Pasteur *et al*, 2010)

Recently there has been a new European Consensus on the definition of an exacerbation, primarily produced for research purposes. It states an exacerbation is when a patient exhibits deterioration in three of more of the following key symptoms for at least 48hours: cough; sputum volume and/or consistency; sputum purulence; breathlessness and/or exercise tolerance; fatigue and/or malaise; haemoptysis AND a clinician determines that a change in bronchiectasis treatment is required (Hill *et al*, 2017).

1.7 Sputum microbiology

The lungs are not a sterile environment and studies suggest 75% of patients with bronchiectasis are colonised with potentially pathogenic microorganisms (Chalmers *et al*, 2012). Colonisation is a term defined by the BTS as the isolation of a pathogen on at least ‘3 separate occasions over a period of at least 3 months and at least two isolates 3 months apart over a year (Pasteur *et al*, 2010). Colonisation can therefore become a chronic state of normality for a patient and does not necessitate treatment, unlike infection when the patient feels unwell with symptoms of an exacerbation (see above) usually in addition to fever, fatigue and systemic upset. The commonly isolated pathogens include *Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Moraxella catarrhalis, Streptococcus pneumoniae* and environmental mycobacteria (Figure 6).

Figure 6.

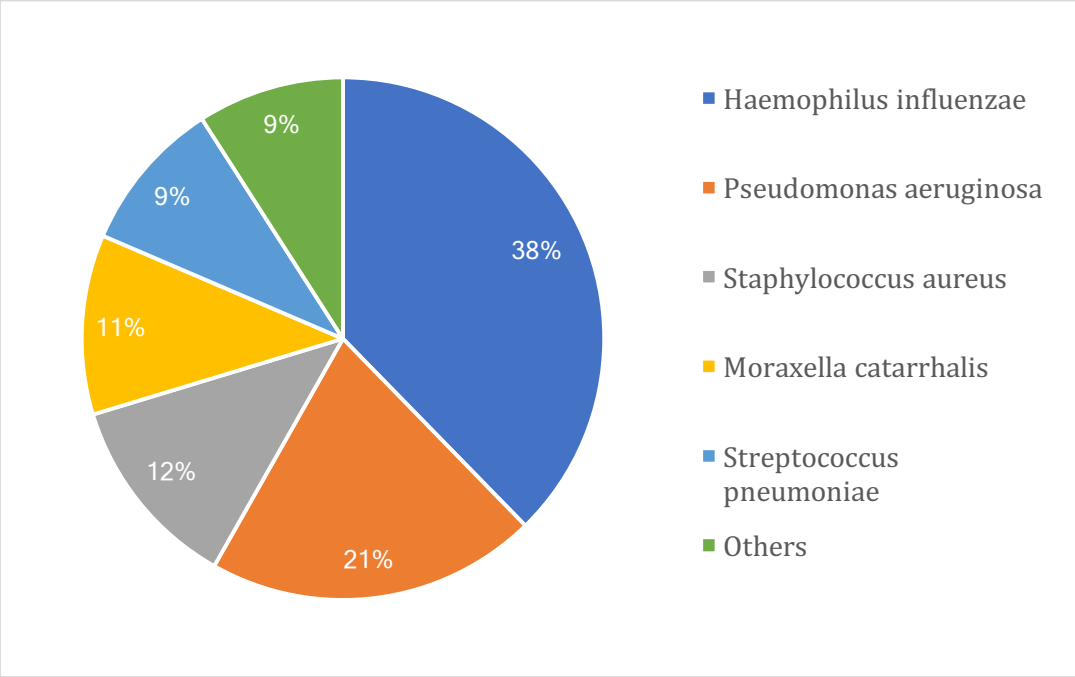


Figure 6 to show the proportion of commonly cultured pathogens in bronchiectasis (Chalmers *et al*, 2012).

1.7.1 *Haemophilus influenzae*

Haemophilus influenzae is the most commonly cultured pathogen in patients with bronchiectasis (Barker *et al*, 2002 Chalmers *et al*, 2012). It is a gram negative coccobacillus, belonging to the *Pasteurellaceae* family. It colonises the nasopharynx as a commensal pathogen in up to 75% of normal adults and is a source of opportunistic infection, primarily causing lower respiratory tract infections (Murphy *et al*, 2001). There are 2 main categories of *Haemophilus influenzae* – capsulated and non-capsulated. The capsule is a major virulence factor that helps the pathogen evade phagocytosis and complement mediated lysis. The non-encapsulated types are thought to be less virile but are still capable of triggering a host inflammatory response. The non-encapsulated strains are often referred to as non-type-able Haemophilus due to the lack of capsular serotypes. The Hib vaccine offered routinely to children is directed against the capsulated HI type B strain and offers no protection against the non-encapsulated types. It has now been recognized that non typeable *H. influenzae* is a major cause of respiratory infection, which tends to be chronic and recurrent and includes sinusitis, otitis media, tonsillitis, pneumonia,

chronic bronchitis and systemic infection (Murphy 2001). Most bronchiectasis patients with chronic non-encapsulated infection can mount a highly effective humoral immune response that prevents systemic infection but there is some suggestion that they favour a predominant Th2 response compared with a predominantly Th1 response in healthy controls which may be important in the pathogenesis of bronchial infection (King *et al*, 2003).

Colonies are small, round and convex and typically appear after 24hrs incubation in aerobic conditions enriched with 5% carbon dioxide at 37 degrees. The species appear negative on Gram staining as spherical, oval or rod shaped cells of less than 1μm diameter. A study by Zhang and colleagues showed non-type-able *Haemophilus influenzae* can last up to 48hours or longer so sputum should be optimally cultured within 3hours of expectoration to maximise chances of isolating the pathogen prior to antibiotic use (Zhang *et al,* 2016).

Haemophilus influenzae produces beta-lactamases, and it is also able to modify its penicillin-binding proteins, so it has gained resistance to the penicillin family of antibiotics. The choice of antibiotic and the duration of antibiotic therapy for treating exacerbations with *H. influenzae* infection are summarised in Table 2.

1.7.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is the second most commonly cultured pathogen in bronchiectasis behind *Haemophilus influenzae* and can be found in 24 - 33% of patients (Pasteur *et al*, 2000). It is a gram negative organism and although not as extensively investigated as it is in cystic fibrosis, it has been associated with increased morbidity and mortality in bronchiectasis. Patients colonised with *Pseudomonas aeruginosa* infection are likely to have more severe disease with a poorer quality of life, have accelerated lung function decline, are at risk of more frequent exacerbations and have increased mortality rates (Ho *et al*, 1998, Wilson *et al,* 1997, Evans *et al*, 1996, Martinez-Garcia *et al,* 2007 & Loebinger *et al,* 2009).

Pseudomonas aeruginosa is a gram negative bacillus that is recognised for its

ubiquitous nature and ability to generate antibiotic resistant properties. It is often a multidrug resistant pathogen associated with severe sepsis including hospital acquired pneumonia and ventilator assisted pneumonia. It is an opportunistic pathogen with immunocompromised patients and those with chronic lung disease and illnesses at risk of serious infection. *P. aeruginosa* can be difficult to treat due to its protective antibiotic evading mechanisms. It can form a biofilm which consists of a community of cells irreversibly attached to a surface and to each other embedded in an extracellular substance exhibiting an altered phenotype (Donlan & Costerton, 2002). Biofilms protect bacteria from adverse environmental factors by one or more mechanisms. These are poorly understood but thought to include the detachment of cells, production of endotoxin, increased resistance to the host immune system and provision of a niche environment for the generation of resistant organisms (Donlan & Costerton, 2002). *P. aeruginosa* can illicit quorum sensing which is the regulation of gene expression to adapt to its environment. It does this by producing small molecules called autoinducers. The extracellular accumulation of these molecules signal the bacteria to alter gene expression to control the expression of virulence factors and hence can rapidly adapt to its environment.

Infection with *P. aeruginosa* has been demonstrated to cause more severe disease. There is therefore some suggestion that eradication of the pathogen when first isolated in sputum should be attempted before colonisation occurs. There are limited randomised controlled trials investigating the benefit and success of eradication. One study evaluating the effect of two weeks of ceftazidime and tobramycin followed by either nebulised tobramycin 300mg (Tobi®) or placebo twice daily for three months in 35 patients with bronchiectasis and a first growth of *P. aeruginosa* found the median time to recurrence of *P. aeruginosa* and the proportion of patients free of *P. aeruginosa* after 12 months follow up was significantly higher in the nebulised tobramycin group. The number of exacerbations, hospital admissions and the number of days in hospital were also significantly lower in the nebulised tobramycin group. Five patients in the nebulised tobramycin group discontinued treatment due to bronchospasm (Orriols *et al,* 2015). The definition of eradication has not yet been defined and it is not known whether eradication is superior to

reducing bacterial load or whether eradication is even achievable. The updated BTS guidelines recommend all patients with new growth of *P. aeruginosa* and clinical deterioration should be offered eradication therapy in the form of ciprofloxacin (500-750mg b.d. for 2 weeks) or second line beta lactam +/- an intravenous aminoglycoside for 2 weeks followed by nebulised colistin, gentamicin or tobramycin for 3 months (Hill *et al*, 2018).

The existence of cross infection of pathogenic strains of *Pseudomonas* between patients has been documented in cystic fibrosis but there is limited evidence in bronchiectasis. De Soyza and colleagues investigated cross infection in a case series of 40 patients. 10 patients were followed up longitudinally for 4yrs. No predominant epidemic strain was found but certain strains found widely within the natural environment were also found in the patients attending the single adult bronchiectasis clinic and thus raising the possibility of environmental acquisition of the pathogen in bronchiectasis patients (Hill *et al,* 2018 & De Soyza *et al*, 2014).

Pujana and colleagues used polymerase chain reaction bacterial fingerprinting to assess cross infection and 2 other studies by Mitchelmore and Hilliam used whole genome sequencing to further explore cross-infection of *P. aeruginosa* in bronchiectasis and all found a very small number of phenotypic heterogeneity within strains from individual patients but overall would not support the idea of significant cross infection with *P. aeruginosa* and suggest that larger longitudinal studies were necessary (Pujana *et al* 1999, Hilliam *et al* 2017 & Mitchelmore *et al* 2016).

There is only 1 oral agent (ciprofloxacin) to treat *P. aeruginosa*, so if resistance or intolerance becomes problematic then treatment options are limited to intravenous regimes. However, in vitro resistance does not necessarily translate to in vivo resistance (Hill *et al,* 2018). New preparations of inhaled ciprofloxacin are being trialled in treatment on/off regimes that deposit the antibiotic at the site of infection thus potentially reducing issues with resistance and systemic side effects (De Soyza *et al*, 2018, Haworth *et al*, 2017, Cartlidge & Hill, 2017). The current recommended treatment regimens for *P. aeruginosa* exacerbation are described in Table 2.

1.7.3 *Streptococcus pneumoniae*

Streptococcus pneumoniae is a gram positive facultative anaerobic member of the genus *Streptococcus*. It usually appears in pairs (diplococci), is haemolytic and non-motile. It can be detected in up to 22% of patients with bronchiectasis (Angrill *et al*, 2002) but is also a common cause of community acquired pneumonia and bacterial meningitis. *S. pneumoniae* can colonise the nasal cavity, sinuses and respiratory tract in healthy individuals but in susceptible patients it can spread and cause disease. It spreads by respiratory droplets via direct contact from person to person. It can also autoinoculate individuals carrying the bacteria in the upper respiratory tract.

The pathogen has a polysaccharide capsule which acts as a virulence factor, helping it to evade phagocytosis and mechanical removal by mucus. The capsule is responsible for helping the pathogen to restrict autolysis by impairing bacterial opsonisation by both the alternative and classical complement pathways, masking subcapsular antigens and prevent binding of complement components to subcapsular components of complement activity.

There are over 90 different serotypes of *S. pneumococcus*, each one with a different polysaccharide capsule. There is a 23-valent pneumococcal vaccine targeted towards 23 different polysaccharide capsules. Widespread vaccination has led to the emergence of mutant strains expressing non-vaccine capsular serotypes and hence emphasises the importance of the capsule in affecting virulence (Hyams *et al*, 2010).

Streptococcus pneumoniae appear as small 1-2mm round colonies that appear in pairs (diplococci), singularly or in short chains and are positive on Gram's staining. The colour of the colonies depends on the degree of encapsulation of the strain of *S. pneumoniae.* It is good practice to culture the bacterial within 3 hours of sputum expectoration to maximise culture results. The choice of antibiotic and the duration of antibiotic therapy for treating exacerbations with *S. pneumoniae* infection are summarised in Table 2.

1.7.4 *Staphylococcus aureus*

Staphylococcus aureus is not as commonly cultured in bronchiectasis as some of the other pathogens already mentioned (Cole, 1995). It is more commonly associated with cystic fibrosis and thought to stimulate the initial destructive inflammatory response that allows chronic *Pseudomonas aeruginosa* infection (Shah *et al*, 1999). Subsequently the rates of *S. aureus* culture are less frequent and thought to be due to anti-staphylococcal properties of *P. aeruginosa* (Machan *et al*, 1991). Shah and colleagues conducted a small study in patients with bronchiectasis colonised with *S. aureus* and found patients more likely to have ABPA or variants of cystic fibrosis.
S. aureus is a gram positive, facultative anaerobe, round shaped bacterium of the family *Staphylococcuceae*. Its colonies are creamy-white or appear either singularly, in pairs or more commonly in clusters. It is part of the body's normal flora and usually located on the skin, nose or respiratory tract. As a commensal organism is does not cause disease but can become more invasive and cause a whole range of illnesses. Apart from respiratory infections it can also cause skin infections including abscesses, cellulitis, impetigo and osteomyelitis, endocarditis and meningitis to name but a few.

The treatment and duration of exacerbations caused by *S. aureus* are outlined in Table 2. More recently bacteraemia caused by this organism can be more concerning due to the increasing resistance patterns observed. Most strains are sensitive to methicillin (methicillin sensitive *Staphylococcus aureus*, MSSA) and therefore to more commonly used antibiotics but there is an increasing incidence of methicillin resistant *Staphylococcus aureus* (MRSA). Even more worryingly there has been an increase in hospital infections with vancomycin resistant *Staphylococcus aureus* and so these patients are usually isolated to contain the spread of resistant mutant strains.

1.7.5 *Moraxella catarrhalis*

Moraxella catarrhalis is an unencapsulated gram negative aerobic bacterium that appears as a diplococcus. Colonies are often small and yellow. *M. catarrhalis* can cause infections of the respiratory tract including bronchiectasis exacerbations, bronchopneumonia, COPD exacerbations as well as sinusitis, laryngitis, otitis media and infections of the eye, central nervous system and joints.

M. catarrhalis is more commonly acquired in childhood and can be present in up to 75% of children as a resident commensal in the nasopharynx (Murphy *et al,* 2013). can be isolated in up to 20% of patients with bronchiectasis and its persistence within the respiratory system can be attributed to multiple factors. It can bind to host epithelial cells by its long range pili and outer membrane proteins including the trimeric autotransporters that allow short range binding. It can invade host cells and by remaining intracellularly can evade the host immune response. It is a hardy organism and can compete with commensal flora and exist under challenging nutritional conditions with the formation of micro-colonies and biofilms and can withstand the effects of human serum (de Vries *et al*, 2009).

M. catarrhalis has rapidly acquired and spread beta-lactamase in the last 2-3 decades and now clinical isolates appear to be resistant to one or more beta-lactam containing antibiotic in 95% of cases (Johnson *et al,* 2003). It has also been suggested that *M. catarrhalis* is also able to protect concomitant colonising pathogens from the effects of beta lactam containing antibiotics (Budhani & Struthers, 2008). There are currently no vaccines directed towards *M. catarrhalis* but several outer membrane proteins are under investigation as potential vaccine antigens. The treatment and duration of antibiotics for exacerbations caused by *M. catarrhalis* are outlined in Table 2.

1.7.6 Other enteric gram negative organisms

Other enteric gram negative pathogens including *Klebsiella, Enterobacteriacea* are of interest in bronchiectasis and studies have shown patients can be colonised with these potentially pathogenic microorganisms in the stable state (Angrill *et al*, 2001). They found patients colonised with these pathogens had an exaggerated inflammatory response with a clear association with bronchial bacterial load. They found poor correlations between bronchial and systemic inflammation but Chalmers and colleagues in 2013 demonstrated that increasing bacterial load was associated with increased bronchial and systemic inflammation which in turn led to an increased risk of further exacerbations (Chalmers *et al,* 2012). Commonly prescribed antibiotics for these pathogens are outlined in Table 2.

1.7.7 Table of commonly prescribed antibiotics for different microbial pathogens.

Table 2.

Pathogen	1st line treatment	Length of treatment	2nd line treatment	Length of treatment
Haemophilus influenzae –β lactamase negative	Amoxicillin 500mg TDS or Amoxicillin 1G TDS or Amoxicillin 3G BD	14 days	Doxycycline 100mg BD or Ciprofloxacin 500mg or 750mg BD or Ceftriaxone 2G OD (IV)	14 days
Haemophilus influenzae -β lactamase positive	Amoxicillin with clavulanic acid 625mg TDS	14 days	Doxycycline 100mg BD or Ciprofloxacin 500mg or 750mg BD or Ceftriaxone 2G OD (IV)	14 days
Pseudomonas aeruginosa	Ciprofloxacin 500mg or 750mg BD	14 days	Monotherapy: intravenous Ceftazidime 2G TDS or Piperacillin with tazobactam 4.5G TDS or Aztreonam 2G TDS or Meropenem 2G TDS Combination therapy: The above can be combined with gentamicin or tobramycin or colistin	14 days
Streptococcus	Amoxicillin	14 days	Doxycycline 100mg	14 days

pneumoniae	500mg TDS		BD	
Staphylococcus aureus -Methicillin sensitive	Flucloxacillin 500mg QDS	14 days	Clarithromycin 500mg BD or Doxycycline 100mg BD or Amoxicillin with clavulanic acid 625mg TDS	14 days
Staphylococcus aureus -Methicillin resistant	Doxycycline 100mg BD or Rifampicin (<50kg) 450mg OD Rifampicin (>50kg) 600mg OD or Trimethoprim 200mg BD Or intravenous: Vancomycin 1G BD Or Teicoplanin 400mg OD	14 days 14 days	Third line Linezolid 600mg BD Linezolid 600mg BD	14 days 14 days
Moraxella catarrhalis	Amoxicillin with clavulanic acid	14 days	Clarithromycin 500mg BD or	14 days

	625mg TDS		Doxycycline 100mg BD or Ciprofloxacin 500mg or 750mg BD	
Coliforms e.g. Klebsiella, Enterobacter	Ciprofloxacin 500mg or 750mg BD	14 days	Ceftriaxone 2G OD (IV)	14 days
Mixed normal flora (respiratory commensals)	Amoxicillin 500mg TDS	14 days	Clarithromycin 500mg BD	14 days

Table 2. 1st and 2nd line antibiotic regimes to treat commonly cultured pathogens in bronchiectasis.

1.8 Epidemiology

Bronchiectasis is a chronic respiratory condition that is diagnosed on high resolution CT scan (HRCT). HRCT is increasingly commonly used as a diagnostic tool as it becomes more widely available and so the incidence of bronchiectasis increases. There is a peak age of onset in adults in the mid-50s with more severe disease associated with increasing age (Chandrasekaran *et al*, 2018). In an ageing population, therefore, the prevalence of bronchiectasis will increase. There is a paucity of studies informing on the prevalence and incidence of bronchiectasis in the United Kingdom.

Until the last decade it was widely believe the incidence of bronchiectasis was decreasing. It was thought to be an illness of the past due to improvement of effective treatment of childhood respiratory conditions, prevention of childhood respiratory conditions- particularly whooping cough and measles through vaccination, and the now effective eradication treatment regimens for pulmonary manifestations of *Mycobacterium tuberculosis* infection.

More recent data from Germany, USA and the UK have suggested there is an increasing prevalence of bronchiectasis with increasing hospitalisation and mortality (Seitz *et al*, 2007, Ringhausen *et al,* 2013 & Robert *et al,* 2013). The first large study investigating incidence and prevalence in the United Kingdom was performed by Quint and colleagues (Quint *et al*, 2016). They investigated prevalence, incidence and mortality over a nine-year period using a Clinical Practice Research Datalink (CPRD) – a large computerised database with longitudinal anonymised medical records in primary care. They found the incidence of bronchiectasis to be higher in women, something that has been long recognised but the cause for which remains unknown. They reported incidence levels increasing from 21.2/100,000 person-years in 2004 to 35.2/100,000 person-years in 2013 with a point prevalence increasing to 566.1/100,000 in 2013. The incidence in men also similarly rose from 18.2/100,000 person-years in 2004 to 26.9/100,000 person-years in 2013 with a point prevalence increasing to 485.5/100,000 in 2013. The incidence of bronchiectasis in both men and women increased with increasing age except in those that were over 80yrs old in both gender groups where the incidence was lower than in the age 70-79yr old age groups. The prevalence also increased with each increase in age decade remaining uncommon in the under-40s but up to 1% of the population in the elderly (aged ≥70yrs old) in 2013. The cause of increasing incidence and increased mortality is not known. It may be due to a change in the prevalence of causes of bronchiectasis i.e. less childhood infections but increased investigation of respiratory conditions in the older populations or the improved diagnosis rates with the use of CT or the improvement in diagnostic labelling with respect to other associated respiratory conditions.

Quint and colleagues also investigated mortality rates in 11,862 individuals with bronchiectasis in England and Wales and showed mortality to be double that of the general population with age-adjusted mortality in women of 1437.7/100,000 compared with 635.9/100,000 of the general population. Similarly, men with bronchiectasis had an age-adjusted mortality rate of 1914.6/100,000 compared with 895.2/100,000. Bronchiectasis can co-exist or be associated with other chronic lung diseases such as COPD and asthma as well as other non-respiratory conditions such

as rheumatoid arthritis. Data on the co-existence of bronchiectasis is currently lacking. The cause of increased mortality is unknown and it is not known as to whether mortality is due to complications from co-existing respiratory or other diseases or whether it results from bronchiectasis itself. Further studies are needed to assess the impact of comorbidities on rates or mortality and the mechanisms of increased mortality in patients with bronchiectasis.

The gender difference present in bronchiectasis has long since been reported with more women diagnosed with bronchiectasis than men (Pasteur *et al*, 2000, Nicotra *et al*, 1995, Weycker *et al*, 2005 and Shoemark *et al*, 2007). Again, the exact cause for this is unknown but several hypotheses have been postulated including sociological reasons; women are more likely to seek medical attention than men, women are more likely to be the primary carer for young children and therefore more likely to be exposed to repeated viral and bacterial infections and biological reasons; reduced muscle mass and strength would lessen the degree of chest expansion and therefore collapse compared to men, hormones present in the luteal phase of the menstrual cycle are thought to have an effect on airway reactivity and some diseases that are associated or causative of bronchiectasis such as rheumatoid arthritis and other connective tissue diseases are more common in women (Morrissey & Harper, 2004). Further work investigating the gender differences in patients with bronchiectasis is needed.

1.9 Diagnosis

1.9.1 Chest radiographs (Chest x-ray)

Upon first recognition of the condition, the diagnosis was predominantly a clinical one as chest radiographs were insensitive for diagnosing bronchiectasis. The chest x-ray is usually normal unless patients have severe bronchiectasis and therefore a normal x-ray cannot exclude bronchiectasis. Characteristic abnormalities on chest x-ray include crowding of the bronchi, parallel tram lines caused by the thickening and dilatation of bronchial walls, ring shadows represent end on dilated bronchi often associated with cystic bronchiectasis when 2cm or more in diameter and oligaemia resultant of lower pulmonary artery pressures. Despite its poor sensitivity

for bronchiectasis and its poor inter – observer agreement, the chest x-ray is often the first imaging investigation performed as digital acquisition devices can provide images with improved visualisation of bronchiectatic airways behind the heart with the added potential of radiation dose reduction (Young *et al* 1991).

Chest radiographs also have poor specificity for bronchiectasis with patient diagnosed with chronic obstructive pulmonary disease (COPD) also often displaying signs of thickened and dilated airways (tramlines and ring shadows) in hyper-inflated lungs on chest x-ray. These changes can also be encountered in patients with asthma and lower respiratory tract infections (Lynch *et al*, 1993 & Cobientz *et al*, 1991). The British Thoracic Guidelines recommends a baseline chest radiograph for all patients but does not recommend its routine use in monitoring patients unless clinically indicated i.e. presence of new symptoms and even then, there is poor correlation between infective exacerbations and radiographic (Pasteur *et al*, 2010).

1.9.2 Bronchograms

In the 1920s a lipiodol substance was injected into the bronchopulmonary tree initially intra-tracheally and later via bronchoscopy immediately followed by chest radiograph to produce a bronchogram (Sicard *et al*, 1922 & Sergeant *et al*, 1923). This method allowed for more objective detection of bronchial dilatation but was not without its risks of allergy to contrast or anaesthetic and impairment of ventilation and diffusion, albeit temporarily. This imaging modality is one of the past now and has been superseded with more advanced imaging modalities such as computer tomography.

1.9.3 High resolution computer tomography (HRCT) – Figure 7.

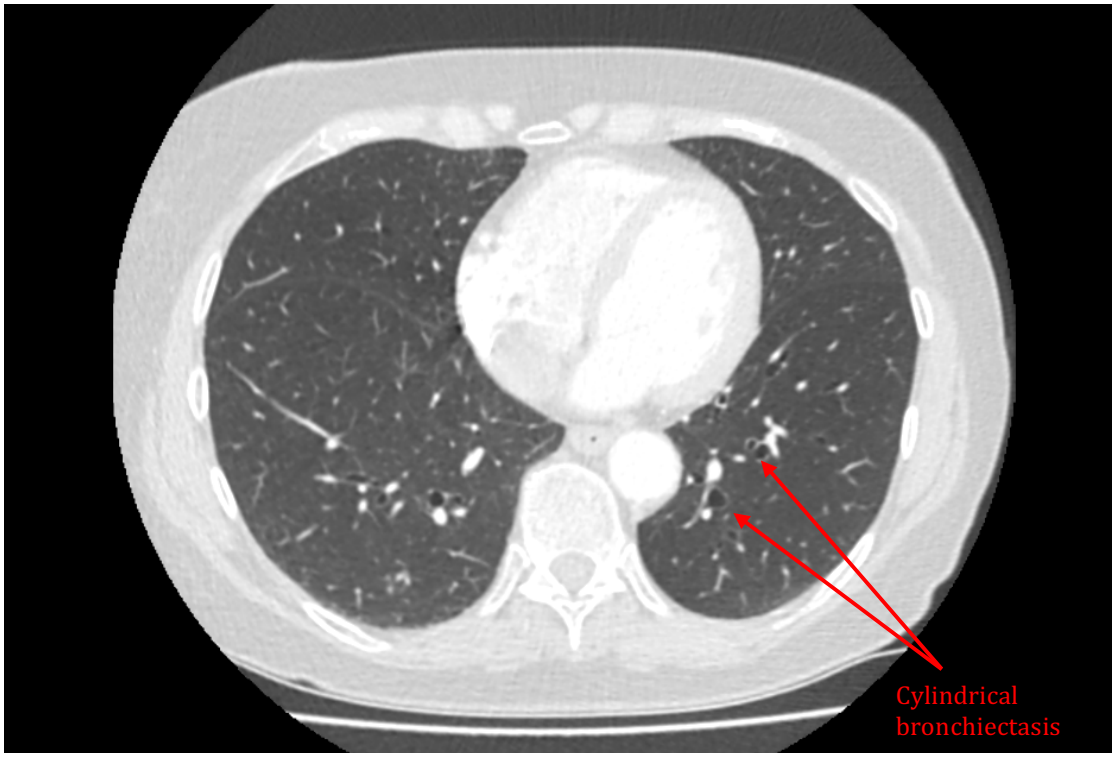
Computer tomography (CT) is the gold standard imaging modality for the diagnosis and monitoring of bronchiectasis. The CT features are quite specific but the earliest abnormalities can be mild and merge with normality. Further confounders include age and cigarette smoking – both of which can cause bronchial abnormalities. One of the earliest signs of suppurative lung disease is bronchial wall thickening but this can be difficult to define and can also be seen in asthma and COPD so diagnosis of early or mild bronchiectasis can be contentious.

The characteristics of bronchiectatic airways on CT scan were first described by Naidich and colleagues to include: air-fluid levels, distended bronchi, linear arrays or clusters of cysts, dilated bronchi in the periphery of the lung and bronchial wall thickening (Naidich *et al*, 1982). Other radiological features that may be present in addition to those originally described by Naidich include emphysema, interlobular septal thickening (Sibtain *et al*, 2005) and a mosaic attenuation pattern due to small airways disease (Hansell *et al*, 1994).

Reasons for poorer sensitivity with standard CT were explored and issues such as motion artefact (transmitted cardiac pulsations could mimic cystic bronchiectasis) and variations of disease such as mucoid impaction altering classical appearances of dilated bronchi were thought to contribute (Tarver *et al*, 1988). Sensitivity improved with the development of high resolution CT (HRCT) assessment of the bronchi in bronchiectasis and it was also proven to have minimal inter- and intra-observer variation with good reproducibility of bronchial wall circumference (Desai *et al*, 1994). The BTS guidelines recommend the use of HRCT with the patient lying supine with breath holding at maximal inspiration. Standard protocol for scanning should be narrow collimation of 1.0mm slices taken at 10mm intervals to give the simplest and lowest radiation dose HRCT technique (Pasteur *et al*, 2010).

HRCT imaging is now the gold standard investigation for diagnosing bronchiectasis with the defining feature being the presence of bronchial dilatation with the internal diameter of the bronchial lumen measuring greater than that of the adjacent artery often referred to as the signet ring sign, with the degree of bronchial dilatation typically categorised as cylindrical, varicose or cystic (Figure 7). The term cylindrical bronchiectasis is used when airways lie parallel to the plane of section and abnormal dilation is seen by a lack of normal tapering, producing a tramline (cylindrical) or flared appearance. Cylindrical bronchiectasis is the most common morphological pattern of bronchiectasis identified on CT. Constrictions along the length of the bronchiole can be termed varicose bronchiectasis and balloon cysts at the end of bronchioles can termed as cystic bronchiectasis.

Figure 7a.



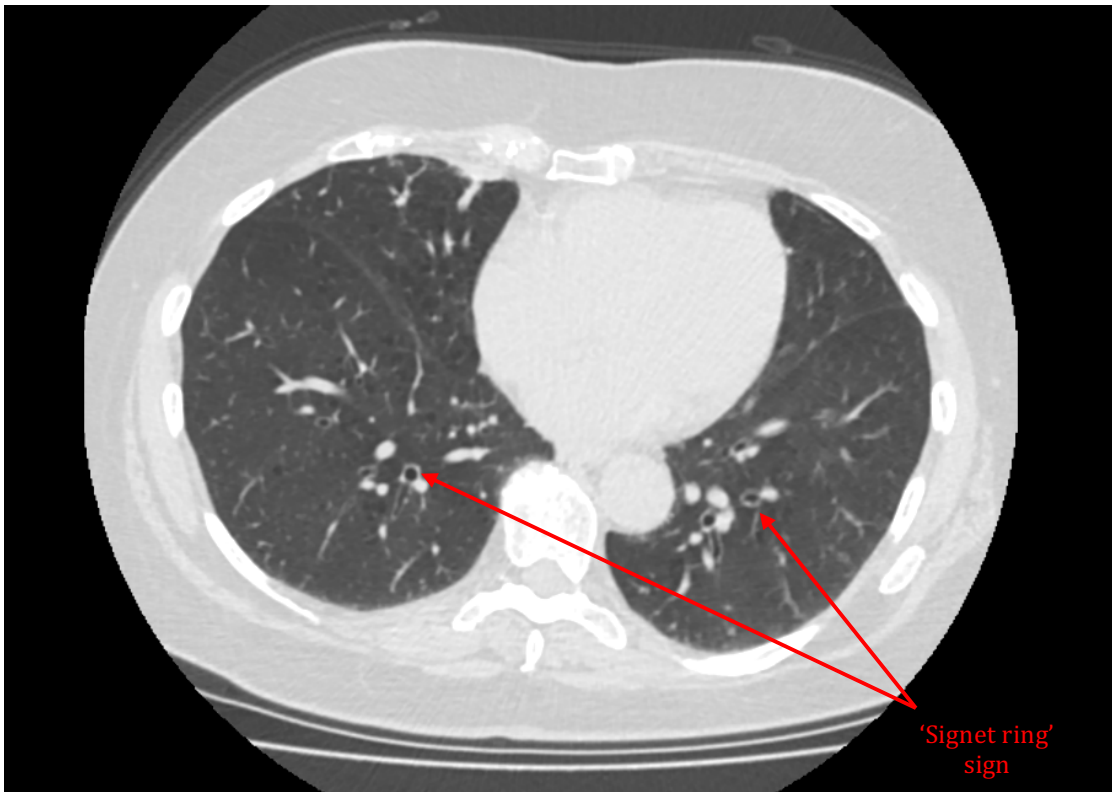


Figure 7b.

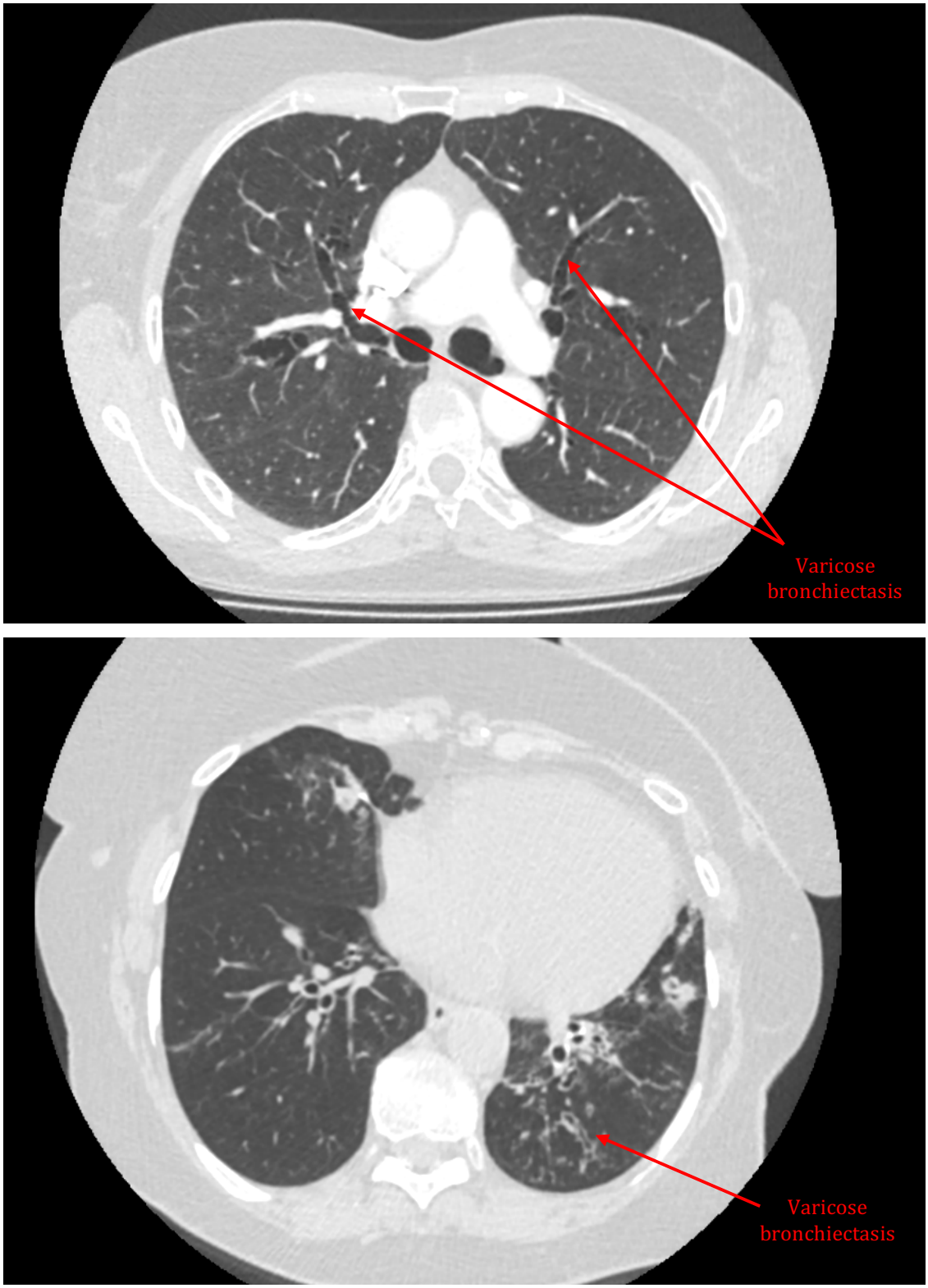


Figure 7c.

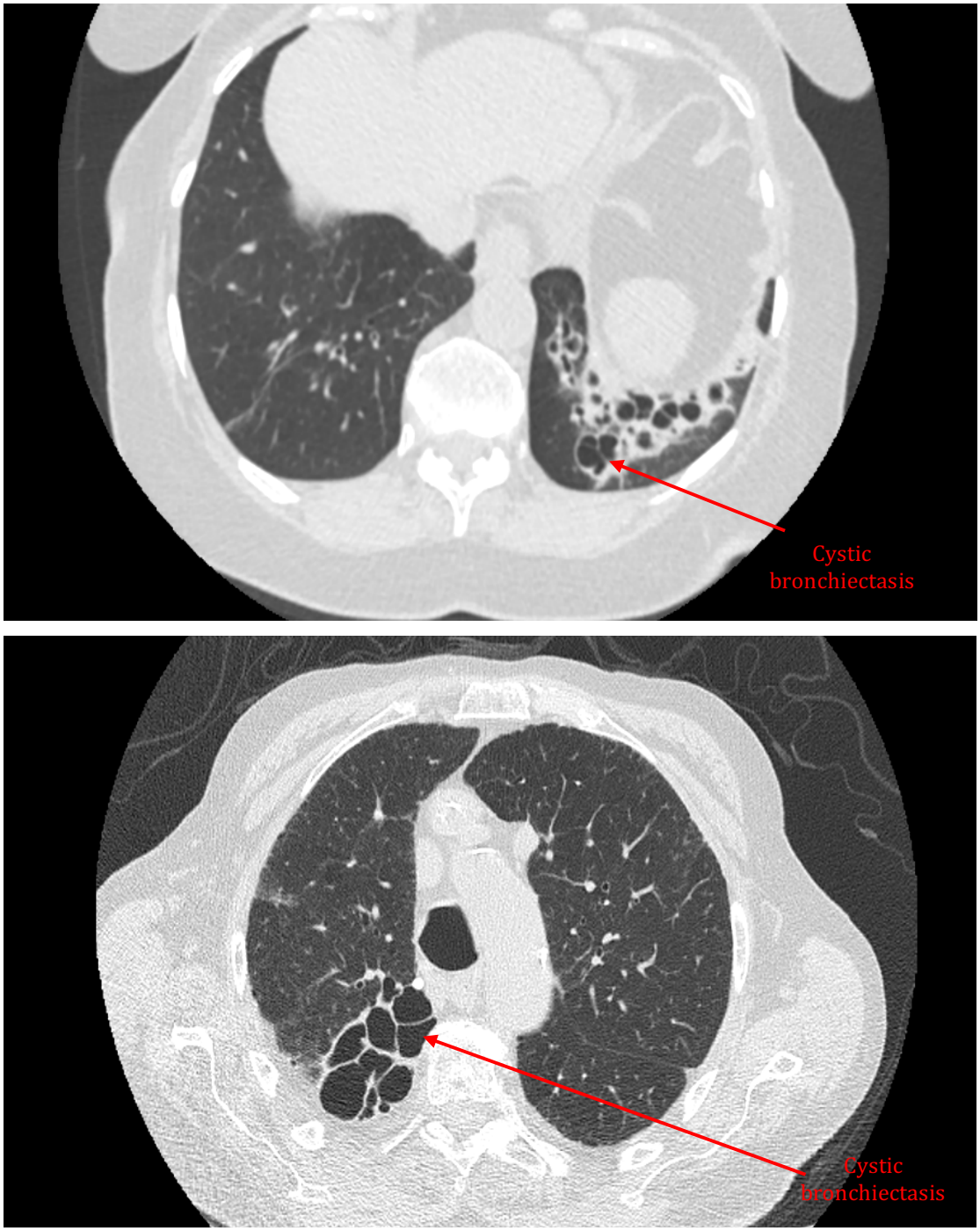


Figure 7. Radiological bronchiectasis a: cylindrical, b: varicose and c: cystic bronchiectasis.

1.9.4 Scoring systems on CT – Bhalla, Reiff and BRICS.

HRCT is now established in the diagnosis of bronchiectasis but there had been no specific validated radiological score to assess severity until recently. Bhalla and colleagues designed a detailed radiological scoring system to quantify structural lung changes in cystic fibrosis using 9 categories: number of lobes affected and segments involved; degree of bronchial dilatation; severity of bronchial wall thickening; extent of mucus plugging; presence or absence of sacculations; presence and number of bullae; presence of emphysema and number of lobes affected; collapse or consolidation; mosaic perfusion; air trapping (Bhalla *et al*, 1991). A modification of this score has been used in research studies (Eshed *et al*, 2007) but has not been validated for use in bronchiectasis. The total range for the Bhalla score is from 3 to 25, with a lower score indicating more severe radiological bronchiectasis. This score was subdivided into mild (16-25), moderate (9-15), and severe (3-8).

Reiff and colleagues subsequently developed a scoring system that described the site, type and extent of bronchiectasis whilst trying to establish if CT imaging could discriminate between idiopathic and other aetiological causes of bronchiectasis but found it to be of limited value. A modified Reiff score has been frequently used in studies gives a score ranging from 0-18 (mild:1-6, moderate: 7-12 and severe:13-18) based on the number of lobes affected (including the lingula) and the severity of bronchial dilatation compared to the adjacent vessel (0: no bronchiectasis; 1: 1-2x; 2: 2-3x; 3: > 3x) (Reiff *et al*, 1995).

Recently the Bronchiectasis Radiologically Indexed CT score (BRICS) was developed by Bedi and colleagues. This score used a multivariate analysis of the Bhalla score and its ability in predicted clinical markers that correlated with disease severity including FEV_1 <50% predicted, sputum purulence and exacerbation requiring hospital admission. Of the 9 categories of the Bhalla score only 2 (degree of bronchial dilatation and number of bronchopulmonary segments containing emphysema) categories were independently associated with the above markers of severity. These two radiological parameters were used to develop the BRICS

simplified score ranging from 0-5 where 1: mild, 2-3: moderate and ≥3: severe disease. The ROC values for the BRICS were 0.79 for percent predicted FEV_1, 0.71 for sputum purulence, and 0.75 for hospital admissions per year and significantly correlated with all 3 clinical severity parameters (p=0.004, p=0.008 and p=0.0001 respectively). The study was conducted in patients with only idiopathic or post-infective bronchiectasis with a previous minimal or none smoking history. It was externally validated (Bedi *et al*, 2018).

Further studies investigating the use of HRCT severity scoring systems in other aetiologies are needed. In addition, long term studies investigating the role of CT and severity scoring as a means of disease monitoring are needed.

1.10 Current management strategies

The pathogenesis of bronchiectasis is poorly understood. Pulmonary pathology shows an excess of neutrophilic inflammation but despite this immune response, over two thirds of patients remain chronically infected with potential pathogenic microorganisms (Angrill *et al*, 2002). The driver for persistent neutrophilic airway inflammation in bronchiectasis is unknown, but infection is considered to play a major role (Chalmers *et al*, 2012). Paradoxically, there is failure of clearance of bacteria from the airways and this leads to a 'vicious cycle' of infection and inflammation in the airways as first described by Cole and colleagues (Cole *et al*, 1989). The neutrophils and bacterial products cause structural damage and perpetuate the vicious cycle. Therapies are needed to break this vicious cycle and indeed antibiotics and anti-inflammatory agents have the potential for this (Sidhu *et al,* 2015). The goals of treatment in this chronic condition are to reduce cough and sputum volume, reduce sputum purulence, reduce the number of chest infections and improve the health-related quality of life.

The management of bronchiectasis with specific aetiological diagnoses of allergic bronchopulmonary aspergillosis, connective tissue disease, rheumatoid arthritis, inflammatory bowel disease, gastro-oesophageal reflux disease, immune

deficiencies and cystic fibrosis should be targeted at the underlying condition and treated as per national guidelines.

1.10.1 The role of vaccinations in bronchiectasis

Influenza vaccinations are currently recommended on an annual basis for patients with chronic lung disease including bronchiectasis (Pasteur *et al*, 2010). There are no randomised controlled trials assessing the effect of influenza vaccination in bronchiectasis (Chang *et al*, 2007) but evidence reported in COPD, another chronic lung disease, in theory may be translatable. Influenza vaccination was found to reduce the number of exacerbations and the number of laboratory confirmed influenza infections in COPD patients 3 or more weeks after vaccination. There was no reported increase in exacerbations immediately after vaccination but there was transiently increased morbidity with arm redness and pain post-injection. Influenza vaccination had no impact on lung function, exercise tolerance, hospitalisation and mortality but the number of studies assessing some parameters (hospital admissions, death rates) was very small and so these conclusions have their limitations (Poole *et al*, 2006).

There is limited evidence supporting the use of 23 valent pneumococcal vaccine to prevent exacerbations in bronchiectasis (Chang *et al*, 2009). One randomised controlled trial in COPD and bronchiectasis patient reported fewer exacerbations when pneumococcal vaccine was administered with influenza vaccines as opposed to influenza alone. There was no change in rates of pneumonia (Furumoto *et al*, 2008). A Cochrane review of pneumococcal vaccines reports the 23-valent vaccine can prevent all cause pneumonia but results different depending on study site, adult population studied and which vaccine was assessed (Moberley *et al*, 2013). The 23-valent pneumococcal vaccine is still offered in the UK, based on the evidence that, for adults between the ages of 65-74 with chronic lung disease, the 23-valent polysaccharide pneumococcal vaccine continues to provide short term protection against invasive pneumococcal disease and remains cost effective (Andrews *et al*, 2012, JCVI 2012). The updated bronchiectasis guideline group recommends the pneumococcal vaccine is offered to all patients with bronchiectasis.

1.11 Management of exacerbations

1.11.1 Role of oral antibiotics in exacerbations

The British Thoracic Society (BTS) recommend sending sputum for bacteriological analysis but to start empirical antibiotics immediately for an exacerbation (Pasteur *et al*, 2010). The antibiotic recommended should be based on prior sputum microbiology but if no previous history is available, empirical amoxicillin (or clarithromycin in cases of penicillin allergy) should be started in addition to other supportive therapy. If patients fail to respond, then antibiotics could be amended based on the pathogen isolated and the antimicrobial sensitivities – Table 2. These recommendations are based predominantly on expert opinion (grade D).

The evidence for oral antibiotic use in exacerbations is lacking, and even more so is the evidence for any particular agent. This may be due to the fact administration of antibiotics is so routine in treating chest infections. In an open labelled study, Hill *et al* investigated the response to oral antibiotics based on sputum purulence. All patients with mucoid (N=7) or mucopurulent (N=7) sputum had improved sputum purulence and/or clinical improvement when treated for an exacerbation with low dose amoxicillin 250mg t.d.s. for 14 days, compared to 16% in those with persistently purulent sputum (N=19). Of those that had treatment failure, 58% then responded to high dose amoxicillin 3g b.d for 14 days. This study demonstrates that higher doses of prolonged antibiotics may be needed for patients with more severe bronchiectasis.

Chan *et al* (1996) and Lam *et al* (1989) investigated the use of quinolone antibiotics versus amoxicillin and reported better clinical and microbiological responses with the quinolones. These cohorts of patients had *Pseudomonas aeruginosa* in 41% and up to 32% respectively in the baseline sputum microbiology and further confirms the need for targeted antibiotic use.

1.11.2 Inhaled antibiotics in exacerbations

Traditionally exacerbations are treated with oral or intravenous antibiotics. The study by Bilton and colleagues studied the effect of adding nebulised tobramycin

300mg B.D. to a 2 week course of oral ciprofloxacin for exacerbations of bronchiectasis due to *Pseudomonas aeruginosa*[10]. They reported significant reductions in bacterial load in the treatment arm at day 7 which was maintained at the end of treatment (day 14) but with increased wheeze. There was no difference in clinical outcomes at day 14 or day 21. There is currently no recommendation to treat exacerbations with inhaled antibiotics (Bilton *et al*, 2006).

1.11.3 Intravenous antibiotics in exacerbations

The British Thoracic Society recommend the use of intravenous antibiotics when: the patient is unwell and requires hospital admission, if resistant organisms are not responsive to oral antibiotics or if patients fail to respond to oral therapy (usually in patients infected with *Pseudomonas aeruginosa* or other enteric Gram-negative organisms) (Pasteur *et al*, 2010). Evidence comparing oral with intravenous antibiotics is lacking and so this recommendation is only based on grade D evidence.

Two small studies have been conducted with bronchiectasis patients investigating intravenous antibiotics but did not show any superiority in using intravenous antibiotics when compared with oral antibiotics (Levofloxacin 300mg B.D. versus Ceftazidime 1g T.D.S. (Tsang *et al,* 1999b) and amoxicillin 500mg T.D.S versus amoxicillin+clavulinc acid 750mg T.D.S versus amoxicillin+clavulanic acid 1.2g IV T.D.S (Mehta *et al*, 1991)) but highlighted the need for targeted antibiotic use.

1.12 Stable state therapy

1.12.1 Chest physiotherapy

Clearance of pulmonary secretions is impaired in bronchiectasis. The expulsion of such stagnant secretions that are prone to infection is a key part of managing patients with bronchiectasis and is usually done with the aid of chest physiotherapy. There are different techniques of chest physiotherapy and these include the Active Cycle of Breathing Technique (ACBT), positive airway pressure adjuncts, oscillating positive airway pressure devices and postural drainage techniques.

Murray and colleagues demonstrated that twice daily chest physiotherapy improved volume of daily sputum expectorated, improved incremental shuttle walk test distance walked and cough related quality of life as assessed by the Leicester Cough Questionnaire (Murray *et al*, 2009b). A Cochrane review identified 7 small studies investigating different airway clearance techniques (ACT) in stable patients over a short-term (single sessions or 15-21days) and long-term (6months) period. It reported the effect of ACT on frequency of exacerbations is unknown and none of the included studies commented on time to next exacerbation or severity of exacerbations. There was low quality evidence to support that sputum expectoration is increased, quality of life is improved, some improvement in lung function, symptoms of breathlessness, cough and sputum production (Lee *et al*, 2015). The lack of randomised controlled trials comparing different techniques and the impact of ACT on patients experiencing an exacerbation mean no single technique of physiotherapy has been shown to be superior but the BTS guidelines recommend all patients with bronchiectasis should be referred to a dedicated respiratory physiotherapist (Pasteur *et al*, 2010) and a physiotherapy plan tailored to the individual's needs and abilities should be suggested to help ensure compliance (Smith *et al*, 2017).

Pulmonary rehabilitation and exercise training programmes have also been shown to be beneficial in several small studies with improvements in functional capacity, improvement in reported dyspnoea (Zanini *et al*, 2015 & Lee *et al*, 2014), health related quality of life (Mandal *et al*, 2012, Ong *et al*, 2011), fewer exacerbations with longer time to first exacerbation (Lee *et al*, 2014) but effects were not sustained at follow up visits after the rehabilitation programmes ended (Lee *et al*, 2014 & Newall *et al*, 2005). The BTS guidelines recommend pulmonary rehabilitation should be offered to patients that are functionally limited by breathlessness (Hill *et al*, 2018).

Inspiratory muscle training (IMT) has been investigated by Newall and colleagues in a small (n=32) randomised controlled trial. Patients were randomised to either an 8 week programme of pulmonary rehabilitation (PR) with IMT or 8 weeks of

pulmonary rehabilitation with sham IMT or to the no intervention control group (Newall *et al*, 2005). They reported functional capacity improved in both intervention groups but quality of life was significantly better in the 8 weeks PR+IMT group. Exercise capacity deteriorated in the PR+sham group at the 3 month follow up but was maintained in the PR+IMT arm. Therefore IMT alone was not reported to increase exercise capacity or quality of life but may be useful in enhancing the longevity of the training effects. IMT should therefore be considered in conjunction with pulmonary rehabilitation for patients with bronchiectasis that are functionally limited by dyspnoea (Hill *et al,* 2018).

31.12.2 Role of bronchodilators

Prevalence of bronchiectasis in patients with COPD has been reported as high as 69% and 43% in patients with asthma (Chalmers *et al*, 2017b). The BTS guidelines advocates all patients with bronchiectasis should be assessed for airway obstruction and reversibility. Bronchodilator therapy with β_2 agonists +/- anticholinergics should be instituted where symptoms or lung function improves with therapy (Pasteur *et al,* 2010). There are no randomised controlled trials assessing bronchodilator therapy (Franco *et al,* 2003 & Lasserson *et al*, 2001) but in a small study (n=24) patients were given β_2 agonist MDI treatment followed by β_2 agonist nebulised therapy and then 24hrs later were given anticholinergic MDI treatment followed by anticholinergic nebulised therapy. 46% of patients were found to respond to one or both treatments with an improvement in FEV_1 ≥15% (Abu Hassan *et al*, 1999). There were similar responses to the β_2 agonist (fenoterol) and the anticholinergic (ipratropium bromide). A randomised trial by Martinez and colleagues in 40 patients investigated combination therapy with formoterol-budesonide 18/640mg versus budesonide 1600mg alone for 3months. There was a significant improvement in quality of life as assessed by SGRQ by 5.3units (p=0.0006), improvement in dyspnoea, cough and reduction in the number of rescue b_2 agonist inhalations with the combination arm. There was no difference in lung function, sputum microbiology or exacerbation rate but fewer side effects with combination therapy, especially those associated with inhaled steroids (Martinez *et al*, 2012).

1.12.3 Role of muco-active therapies

Regular chest clearance is regarded as key for all patients with clinically significant bronchiectasis. To aid this, there has been interest in treatments that improve mucociliary clearance and sputum expectoration. Hypertonic saline, mannitol, carbocysteine and DNase are the most studied agents but there are limited phase III trials in non-cystic fibrosis bronchiectasis patients. Hypertonic saline and mannitol are hyperosmolar agents that improve airway hydration by inducing a liquid flux to the airway surface. This in turn helps to mobilise sputum.

Hypertonic saline has been investigated in several small trials in bronchiectasis. A study in 8 patients demonstrated the effect of isotonic 0.9% saline on sputum mobilisation by reporting increased sputum expectoration when using immediately prior to chest physiotherapy when compared to chest physiotherapy alone (Sutton *et al*, 1988). In a 4-way crossover study, 24 patients were randomised to one of the four arms: i) active cycle breathing technique (ACBT); ii) nebulised terbutaline and ACBT; iii) nebulised terbutaline, nebulised isotonic saline (IS) then ACBT; and iv) nebulised terbutaline, nebulised hypertonic 7% saline (HTS) then ACBT. The authors reported improved ease of sputum expectoration, reduced sputum viscosity and a small improvement in lung function with the use of inhaled HTS (7%) (Kellet *et al,* 2005). A second randomised single-blind study by the same group over a 3month period in 28 patients comparing 7% HTS with 0.9% IS saline reported HTS had a significant improvement in quality of life as assessed by the St George's Respiratory Questionnaire ($p<0.05$) and significant increase in FEV_1 by 15% from baseline versus 1.8% with 0.9% IS ($p<0.01$) (Kellet *et al*, 2011).

A randomised controlled trial investigating the effects of 6% HTS over 12 months was conducted, where 40 patients were randomised to receiving either once daily 6% HTS or 0.9% IS (Nicolson *et al,* 2012). The authors found similar significant improvements in quality of life and lung function (FEV_1 and FEF_{25-75}) for both groups at 6 months but the improvement in FEV_1 was not sustained at 12 months. There was a significant reduction in sputum colonisation from 60% in the IS group and 55% of HTS group at baseline to 15% in both groups at 12 months. There was

no difference between the groups in any of the end points, including exacerbation frequency.

The lack of large randomised trials on hypertonic saline preclude its recommendation for regular use in patients with bronchiectasis as there is some evidence to that suggests HTS may improve sputum clearance and quality of life but it remains unclear if this benefit is superior to that of IS use. The BTS guidelines suggest a trial of muco-active therapy could be considered in patients experiencing difficulty expectorating (Hill *et al,* 2018).

Mannitol is a naturally occurring sugar thought to alter the osmotic gradient in the airways and thus change the properties of mannitol making it easier to expectorate. Inhaled mannitol has also been investigated with several small studies with mixed results of improved sputum expectoration (Daviskas *et al*, 1999 & Daviskas *et al,* 2008), improvement in quality of life (Daviskas *et al*, 2003), no change in lung function and that it was well tolerated despite inducing cough (Daviskas *et al*, 2003, 2005, 2008). In 2014, Bilton and colleagues conducted a larger multicentre double-blind randomised controlled trial with 461 patients with excess sputum production and 2 or more exacerbations in the last 12 months were randomised to either 400mg mannitol b.d or low dose mannitol control b.d. (Bilton *et al*, 2014). They found the exacerbation rate did not significantly reduce on 400mg mannitol (p=0.3) but the time to next exacerbation did significantly lengthen (165days versus 124days, p=0.02). Quality of life as assessed by the St George's Respiratory Questionnaire was also found to improve with mannitol (p=0.046) but there was no benefit in lung function or sputum bacteriological properties. Inhaled mannitol was well tolerated with similar adverse events experienced in both the mannitol and control group. As this mannitol study failed to reach its primary endpoint, it is unlikely to be used routinely in clinical practice.

Mucolytics such as DNase, carbocysteine and bromhexine have also been investigated to enhance mucociliary clearance by changing the physiochemical properties of sputum. This reduction in sputum viscosity should allow easier

mobilisation. A Cochrane review identified only 3 studies (described below) assessing mucolytics in bronchiectasis patients (Crockett *et al*, 2001). Mucolytics remain a promising prospect for treatment but randomised controlled data is lacking to support any recommendations for regular use.

The first study by Olivieri and colleagues, assessed bromhexine 30mg t.d.s versus placebo. They noted an improvement in 'difficulty in expectoration', an increase in sputum production and a significant reduction in cough. There was no change in lung function (Olivieri *et al*, 1991). Wills *et al* conducted a double blind randomised control trial studying human recombinant DNase (rhDNase) 2.5mg twice daily, 2.5mg once daily, placebo twice daily or placebo once daily in 21 patients with bronchiectasis (Wills *et al*, 1996). They failed to show any improvement in lung function, dyspnoea or quality of life at the higher dose but did report a significant improvement in dyspnoea with low dose rhDNase. Influenza-like symptoms were reported in 4 subjects randomised to high dose rhDNase. O'Donnell and colleagues conducted a large double blind randomised control trial investigating rhDNase versus placebo twice daily for 24 weeks in 349 patients (O'Donnell *et al*, 1998). The authors reported a significant increase in protocol derived and non-protocol derived exacerbation rates when compared with placebo. They also reported a significant reduction in FEV_1 compared with placebo (FEV_1 decline -3.7% change vs -1.7% in placebo group) and as a consequence of this trial DNase is not recommended in the treatment of bronchiectasis (Pasteur *et al*, 2010).

1.12.4 Role of inhaled corticosteroids

A Cochrane review found limited small studies investigating the use of inhaled corticosteroids in bronchiectasis but no large placebo-controlled randomised controlled trials (Kapur *et al*, 2018). The small studies have suggested that inhaled corticosteroids can reduced sputum volume (Tsang *et al*, 2005, Elborn *et al*, 1992) and improve quality of life (Martinez *et al*, 2006) but they have not proven whether long-term treatment has any effect on lung function or exacerbation rates (Tsang *et al*, 1998). In addition, there is some concern with the use of single agent inhaled corticosteroid use in COPD due to the risk of pneumonia (Janson *et al*, 2013). Large

randomised controlled trials assessing benefit versus risk of these treatments must be conducted before their routine use can be recommended (Pasteur *et al*, 2010).

1.12.5 Non-steroidal anti-inflammatories (NSAIDS)

A Cochrane review identified only 1 small randomised double blinded controlled study comparing inhaled indomethacin 2.4micrograms, t.d.s for 14 days in 25 patients with a variety of chronic lung diseases (8 bronchiectasis, 12 chronic bronchitis and 5 diffuse panbronchiolitis) with placebo (Pizzutto *et al*, 2010 & Tamaoki *et al,* 1992). Results showed an improvement in the amount of sputum expectorated and dyspnoea but no change in lung function. A second study of 9 patients with stable bronchiectasis were given 4 weeks of oral 25mg oral indomethacin (t.d.s). Analysis showed no effect on airway bacterial load or sputum chemotaxis but there was marked reduction in peripheral blood neutrophil function with reduced chemotaxis and fibronectin degradation (Llewellyn-Jones *et al*, 1995). Larger randomised controlled trials to assess NSAIDs as potential anti-inflammatory agents are needed before they can be considered for routine treatment.

1.12.6 Statins

Statins are widely used in cardiovascular disease and have been shown to be associated with a 41% reduction in 30-day mortality in patients hospitalized with seasonal influenza. One of the pleotropic effects of statins is their anti-inflammatory property, which could have a role in breaking the vicious cycle of bronchiectasis. To date, only two randomised controlled trials has been conducted in patients with bronchiectasis. 60 patients were randomly allocated to either 80mg Atorvastatin once daily or placebo for 6 months. The authors reported a significant improvement in cough as assessed by the Leicester Cough Questionnaire with the intervention group ($p=0.01$) but also more adverse events ($p=0.02$) (Mandal *et al*, 2014).

A randomised placebo cross over trial investigated atorvastatin 80mg daily for 3 months in patients chronically infected with *P. aeruginosa*. Statin therapy did not improve the primary endpoint for improved cough assessed by the Leicester Cough Questionnaire. There were however significant improvements in the St. George's

Respiratory Questionnaire (5.6 Unit improvement) and a reduction in serum Interleukin-8, Tumour necrosis factor-alpha and Intercellular adhesion molecule-1 in the statin treated group (Mandal *et al*, 2017). Further randomised trials to assess their effect on exacerbation frequency are required.

1.12.7 Others

Because of the excess neutrophil burden in bronchiectasis, future studies are needed to assess novel therapies such as neutrophil elastase (NE) inhibitors and tumour necrosis factor alpha (TNFα) inhibitors but to date there has only been one small study on neutrophil elastase inhibitors and none in TNFα inhibitors.

Stockley *et al* randomised 40 patients to receiving NE inhibitor AZD9668 60mg, b.d. (n=22) or placebo (n=16) for 4 weeks (Stockley *et al*, 2013). They reported a significant improvement in FEV_1 of 100mls, slow vital capacity, plasma interleukin 8 and post waking sputum interleukin 6. There was no significant improvement in quality of life, sputum weight or other lung function tests in the treatment group. AZD9668 seemed to be well tolerated with fewer adverse events reported than in the placebo arm. The most common complaint in the treatment arm was of headache 7/22 compared with 2/16 in the placebo arm. These results are encouraging but larger studies of longer duration are needed.

De Soyza and colleagues investigated CXCR2 antagonist AZD5069 80mg twice daily in a randomised double-blind placebo-controlled parallel-group trial for 28 days in 52 patients with bronchiectasis. CXCR2 is a surface membrane receptor for Interleukin-8 which is partly responsible for neutrophil migration into the lungs. They reported a significant reduction in absolute percentage sputum neutrophil counts with AZD5069, but no difference was seen with the number of exacerbations or serum C-reactive protein levels. There were more discontinuation of treatment with AZD5069 but the compound was overall well tolerated (De Soyza *et al*, 2015). These results are encouraging but larger studies of longer duration are needed.

1.12.8 Role of long term oral antibiotics

Studies have shown exacerbations reduce quality of life. It stands to reason then by reducing the number of exacerbations, quality of life should improve. Long term oral antibiotics have been investigated previously with the rationale that reducing bacterial load burden would reduce inflammation and hence allow the bronchial tree to repair/heal leading to fewer symptoms, exacerbations, and improved quality of life. The first studies were conducted in the 1950s and 1960s and mainly showed tetracyclines improved clinical outcome (reduced sputum volume and purulence), reduce exacerbation frequency but also lead to gastrointestinal side effects in 20-30% or patients (MRC, 1957, Sobel *et al*, 1962 & Cherniack *et al*, 1959). Whilst tetracyclines reduced some bacterial species (*S. pneumoniae, H. influenzae, S. aureus*) it led to an increase in the culture of *Pseudomonas aeruginosa* (Dowling *et al*, 1960). One further study was conducted in 38 patients randomised to either high dose amoxicillin (3g, twice daily for 32weeks) or placebo found high dose amoxicillin led to significant clinical improvement with less severe exacerbations (Currie *et al*, 1990). There have been no large randomised controlled trials investigating the role of long-term non-macrolide oral antibiotics. In view of limited treatments, their use is only recommended in patients experiencing 3 or more exacerbations a year despite optimizing chest clearance techniques and treatment for comorbidities e.g. reflux or rhinosinusitis. Long term oral antibiotics are suitable particularly in patients colonised with potential pathogenic organisms, for example *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*, which have multiple suitable oral antibiotic preparations. The major limiting factor is that such long-term antibiotics have side effects and predominantly diarrhoea (Sobel *et al,* 1962 & Dowling *et al,* 1960). Further randomised controlled studies are required to assess the effect of long-term antibiotic use on the generation of resistance.

1.12.9 Role of long term inhaled antibiotics

Inhaled and nebulised antibiotic therapies target the airways directly and should thereby reduce systemic side effects. Several studies have investigated the role of long term nebulised antibiotics in the stable state and are summarised below. These key studies are also listed in table 3 (Sidhu *et al,* 2014).

Author	Study	N	Antibiotic	Dose & Duration	Microbiology	Results	Interpretation
Lin *et al*, 1997	Randomised	16	Gentamicin Versus 0.45% saline	40mg, b.d, 3 days	Not recorded	The gentamicin group led to a reduction in microbial load, sputum Myeloperoxidase (MPO) levels and sputum volume. There was improved Borg breathlessness score, peak expiratory flow rates and 6 min walk.	Early proof of concept study showing inhaled antibiotics could reduce microbial burden and improve clinical outcomes.
Barker *et al*, 2000	Randomised	74	Tobramycin Versus Placebo	300mg, b.d, 4 weeks on treatment then 2 weeks off	100% *P. aeruginosa*	The tobramycin group at week 4 led to a significant reduction of sputum bacteria by 4.54 log (10) cfu/g with tobramycin with no change in the placebo group, $p<0.01$. Tobramycin subjects also showed a 35% eradication rate at week 6, with no eradication in placebo. Tobramycin group had increased cough, wheeze, chest pain and breathlessness.	Inhaled tobramycin over 4 weeks can eradicate *P. aeruginosa* in 35% - an outcome that is very different to the cystic fibrosis population.
Wilson *et al*, 2013	Randomised	124	Ciprofloxacin (DPI) Versus Placebo	32.5mg, b.d, 28 days	54% *P. aeruginosa* 24% *H. influenzae* 20% *S. aureus* 7% *S. pneumoniae* 6% *M. catarrhalis* 4% *K. pneumoniae* 6% *P. mirabilis* 4% *K. oxytoca*	There was a significant reduction in bacterial density for those on ciprofloxacin DPI with total sputum bacterial load at day 28 - end of treatment (EOT) (-3.62 $\log_{10}$ $CFUg^{-1}$ (range -9.78–5.02 $\log_{10}$ $CFUg^{-1}$)) compared with placebo (-0.27 $\log_{10}$ $CFUg^{-1}$ (range -7.96–5.25 $\log_{10}$ $CFUg^{-1}$)) ($p<0.001$). The counts increased thereafter until they were similar at day 84. 14/40 subjects in the ciprofloxacin DPI group reported pathogen eradication at EOT vs 4/49 in the placebo group ($p=0.001$).	Inhaled ciprofloxacin improved bacterial clearance over 28 days and was well tolerated.

						3/60 in ciprofloxacin vs 3/64 in placebo reported bronchospasm, 0/60 vs 5/64 reported cough and 1/60 vs 2/64 reported haemoptysis. No change in FEV_1 or FVC for either group. No significant difference between the groups in sputum volume or colour.	
Drobnic *et al*, 2005	Randomised crossover study	30	Tobramycin Versus Placebo	300mg, b.d, 6 months	100% *P. aeruginosa*	No effect on overall number of exacerbations, pulmonary function or health status. The tobramycin treated group had a reduced number of and length of hospital admissions ($p<0.05$). Significant reduction in *P. aeruginosa* bacterial load ($p<0.05$). Bronchospasm occurred in 10%. No significant increase in bacterial resistance.	Inhaled tobramycin in patients with *P. aeruginosa* over 6 months led to a reduction in severity of admissions and with no impact on bacterial resistance.
Serisier *et al*, 2013b	Randomised	42	Ciprofloxacin (DRCFI) Versus Placebo	150mg ciprofloxacin for inhalation + 20mg free ciprofloxacin for inhalation, o.d., 24 weeks (3x 28days on/28days off)	100% *P. aeruginosa*	There was a mean reduction in *P. aeruginosa* bacterial density at day 28 of 4.2 $\log_{10}$ CFU/g with DRCFI compared to a mean reduction of 0.08 with placebo ($p=0.002$). Time to first exacerbation was significantly delayed by DRCFI (134 days) when compared with placebo (58days). DRCFI was well tolerated with fewer respiratory related adverse events than placebo. Main treatment related adverse events included nausea, sinusitis, fatigue, headache and abnormal taste. 3 subjects in each arm developed an exacerbation – thought not to be related to treatment.	In per protocol analysis inhaled ciprofloxacin led to a delayed first exacerbation in this 6 month study.

De Soyza *et al*, 2018	Randomised	416	Ciprofloxacin (DPI) versus placebo	32.5mg b.d. for 14 days on/14days off versus placebo OR 32.5mg b.d. for 28 days on/ 28 days off versus placebo	Predefined bacterial culture including *P. aeruginosa*	Ciprofloxacin DPI 14 days on/off significantly prolonged time to first exacerbation *versus* pooled placebo (median time >336 *versus* 186 days; hazard ratio 0.53, 97.5% CI 0.36–0.80; p=0.0005). Ciprofloxacin DPI reduced the frequency of exacerbations compared with matching placebo by 39% (mean number of exacerbations 0.6 *versus* 1.0; incidence rate ratio 0.61, 97.5% CI 0.40–0.91; p=0.0061). There was no significant difference from placebo for the 28 days on/off treatment. The safety profile was favourable.	Cyclical treatment with 14 days on and off with Ciprofloxacin DPI was well tolerated and led to reduced exacerbations and increased time to next exacerbation. The 14 day cyclical regimes were superior to the 28 day cyclical regimes which did not show any significant difference to treatment with placebo.
Aksamit *et al*, 2018	Randomised	521	Ciprofloxacin (DPI) versus placebo	32.5mg b.d. for 14 days on/14days off versus placebo OR 32.5mg b.d. for 28 days on/ 28 days off versus placebo	Predefined sputum bacteria including *P. aeruginosa*	There was a trend towards prolonged time to next exacerbation (ciprofloxacin DPI 14 days on/off: hazard ratio 0.87, 95.1% CI 0.62–1.21; p=0.397; ciprofloxacin DPI 28 days on/off: hazard ratio 0.71, 99.9% CI 0.39–1.27; p=0.051) but this was not statistically significant. There was a trend towards reduced exacerbation frequency in both active ciprofloxacin treatment groups (ciprofloxacin DPI 14 days on/off: incidence rate ratio 0.83, 95.1% CI 0.59–1.17; p=0.2862; ciprofloxacin DPI 28 days on/off: incidence rate ratio 0.55, 99.9% CI 0.30–1.02; p=0.0014. The overall exacerbation rate for the study was (mean±SD 0.6±0.9).	The primary endpoints for this study were not achieved. There was no statistical difference between Ciprofloxacin DPI and placebo.

						Ciprofloxacin DPI was well tolerated.	
Haworth *et al*, 2017	Randomised	582	Ciprofloxacin (ARD - 3150, pulmaquin) nebulised versus placebo	150mg ciprofloxacin for inhalation + 60mg free ciprofloxacin for inhalation, o.d., 48 weeks (6x 28days on/28days off)	100% *P. aeruginosa*	Ciprofloxacin treatment led to a clinically significant increase in the time to next exacerbations requiring antibiotics. There was also a significant reduction in the frequency of protocol defined exacerbations (irrespective of whether antibiotics were prescribed). No difference in time to next protocol defined exacerbation. There was significantly reduced sputum *P. aeruginosa* bacterial density. with ciprofloxacin. Similar rates of adverse evcnts/serious adverse events between the ciprofloxacin and placebo group.	Nebulised Ciprofloxacin in 28 day on/off regime for 6 cycles reduced the number of protocol defined exacerbations but not the time to next protocol defined exacerbation in patients colonised with *P. aeruginosa*. It was well tolerated.
Orriols *et al*, 1999	Randomised open labelled study	15	Ceftazidime + tobramycin Versus Symptomatic treatment	1g, b.d & 100mg b.d, 1 year	100% *P. aeruginosa*	In the active group the mean number of admissions and length of admission were significantly lower than symptomatic treatment. No change in FEV_1 or FVC for overall exacerbation frequency for either group. No significant increase in bacterial resistance.	This small study supports that long term inhaled antibiotics reduce the severity of exacerbations.
Murray *et al*, 2011	Randomised single blinded study	65	Gentamicin Versus 0.9% Saline	80mg, b.d, 1 year	48.1% *P. aeruginosa* 40.7% *H. influenzae* 7.4% *S. aureus* 3.4% *S. pneumoniae*	The gentamicin group led to 1. Increased ETT 95m 2. Leicester Cough Questionnaire 81% had 1.3U improvement or greater vs. 20% placebo 3. SGRQ 82.5% had 4U improvement or greater vs. 19.2% placebo	This 1 year study supports long term inhaled antibiotics reduce total number of exacerbations and time to first exacerbation. Treatment, however, has to be continuous for its ongoing efficacy.

						4. Increased time to next exacerbation (Gentamicin 120d (87-162) vs. Saline 61.5d (20-7-122.7)) 5. Decreased exacerbations (Gent 0(0-1) vs. Saline 1.5(1-2)) 6. No significant effect on antimicrobial resistance There was however in the gentamicin group, 1. No effect 24hr volume, FEV_1, FVC, FEF25/75 2. 21.9% (7 of 32 patients) reported bronchospasm and received adjunctive nebulised β_2 agonist treatment. Despite this, two patients (6%) required withdrawal from the study (one at month 3 and one at month 6) Treatment needs to be continuous for its on-going efficacy.	
Haworth et al, 2014	Randomised, double-blind	144	Colistin versus placebo (0.45% saline)	1million I.U. via ineb b.d, 6months	100% *P. aeruginosa*	Primary endpoint of time to next exacerbation showed no difference between Colistin and placebo (p=0.11). Time to next exacerbation based on adherence recorded by the I-neb was delayed in the Colistin arm; 168days versus 103days (p=0.038). *P.aeruginosa* density reduced after 4 (p=0.001) and 12 weeks (p=0.008). SGRQ scores improved after 26weeks (p=0.006). No Colistin resistant strains of P. aeruginosa	Primary endpoint of time to next exacerbation was not achieved. In treatment adherent patients (took medication more than 80% of the time) there was a significant delay in time to next exacerbation. Overall well tolerated with no issues of resistance with continuous therapy.

						identified, no difference in treatment emergent pathogens between the groups. No difference in FEV_1, sputum weight or adverse events.	
Barker *et al*, 2014	Randomised	266	Aztreonam versus placebo	75mg o.d. for 16 weeks (2x 4weeks on/4 weeks off)	Chronic colonisation with gram negative organisms	Primary endpoint of change from baseline Quality of Life-Bronchiectasis Respiratory Symptoms scores (QOL-B-RSS) was not significantly different between active and placebo arms. There was a statistically significant difference in QOL-B-RSS but the 4.6unit change was not thought to be clinically relevant. In both studies treatment adverse events and discontinuations from treatment were more common in the Aztreonam group.	No improvement in respiratory symptoms; time to first exacerbation not prolonged; more treatment-related adverse events; more discontinuations with treatment than placebo
		274	Aztreonam versus placebo	75mg o.d. for 16 weeks (2x 4weeks on/4 weeks off)	Chronic colonisation with gram negative organisms		
Table 3. Inhaled antibiotic studies in stable bronchiectasis							

The studies to date show encouraging preliminary data that long term inhaled antibiotics can reduce the bacterial burden and improve clinical outcomes (Drobnic *et al*, 2005; Serisier *et al*, 2013b; Orriols *et al*, 1999 & Murray *et al*, 2011). New phase 3 trials have shown in randomised controlled trials that long-term dry powder for inhalation preparations of Ciprofloxacin may have effects of reducing exacerbation frequency and prolonging the time to next exacerbation but results are not consistent. RESPIRE-1 and RESPIRE-2 are identical trials in different cohorts. In RESPIRE-1, De Soyza and colleagues reported increased time to next exacerbation (p=0.0005) and reduced frequency of exacerbations (p=0.0061) with a 14day on/off regime but did not see this effect with the 28day on/off treatment arms (p=0.065 and p=0.89 respectively) (De Soyza *et al*, 2018). 24.5% of all isolates at baseline had increased minimum inhibitory concentrations (MICs) for ciprofloxacin and the number of patients that cultured pathogens with increased MICs during treatment was higher with ciprofloxacin than with placebo (14days: 20.4%, 28days: 26.3% and pooled placebo: 12.3%). If taking into account any pathogens with increased MICs including at baseline and End of Study (8weeks after trial, EOS) the percentages increase to 54%, 53.9% and 36.2% for 14day, 28day and pooled placebo respectively. At EOS, there was less than 10% of elevated MIC pathogens for the ciprofloxacin arms and 2.2% in the placebo arms suggesting resistance off treatment was not maintained. The clinical consequences of elevated MICs require further investigation as subgroup analysis did not show reduced efficacy in those patients with elevated MICs at baseline. RESPIRE-2, Aksamit and colleagues (2018) did not show an increase in time to next exacerbation (14day: p=0.40, 28day: p=0.05) or reduction in exacerbation frequency (14day: p=0.29, 28day: p=0.0014). RESPIRE-2 did not meet its primary endpoints.

2 further phase 3 trials (ORBIT-3 and ORBIT-4) are underway and preliminary results only have been published in abstract format reporting 28day on/off cycles of ciprofloxacin reduced frequency of protocol derived exacerbations but not increased time to next protocol defined exacerbation (Haworth *et al*, 2018). Overall inhaled/nebulised ciprofloxacin appears to be well tolerated.

A meta-analysis by Brodt and colleagues in 12 trials (including most of those listed above in addition to unpublished trials) evaluating the efficacy and safety of inhaled antibiotics in 1264 patients with bronchiectasis reported inhaled antibiotics were more effective at reducing sputum bacterial load, sputum bacterial eradication and reducing exacerbations than placebo or symptomatic treatment (Brodt *et al*, 2014).

There has been a longstanding debate whether these treatments should be 14-day or 28-day, on and off or continuous (Murray *et al,* 2011, Haworth *et al*, 2014, Serisier *et al,* 2013b, De Sozya *et al,* 2018, Aksamit *et al,* 2018, Haworth *et al*, 2017 & Barker *et al,* 2014). The theory for the on-off regimen was to reduce the development of microbial resistance. The long term antibiotic studies to date have not shown that continuous antibiotics lead to bacterial resistance affecting patient care (Drobnic *et al,* 2005; Orriels *et al,* 1999, Murray *et al,* 2011 & Haworth *et al,* 2014). It is likely that inhaled therapy will be predominantly used in patients colonised with more complex pathogens such as *Pseudomonas aeruginosa* where there are limited or no long term suitable oral therapies. The limiting factors to date using these therapies are they have significant cost to their use and are all currently unlicensed. In addition, such therapies can cause bronchospasm and breathlessness and around 10% of patients will have to discontinue therapy despite the addition of inhaled or nebulised bronchodilator therapy because of these symptoms (Barker *et al*, 2000; Drobnic *et al*, 2005 & Murray *et al*, 2011, Brodt *et al*, 2014).

An alternative approach in patients with very severe bronchiectasis is the consideration for regular planned eight weekly courses of intravenous antibiotic therapy. A small study showed that this treatment improved patients' symptoms with reduced antibiotic burden, cough and improved health status and reduction of systemic inflammation (Mandal *et al,* 2013). Randomised controlled trials are however needed to explore this further.

1.12.10 Role of Macrolides

There has been a growing interest in newer therapies for bronchiectasis to break the 'vicious cycle'. These include anti-inflammatory and anti-infective therapies. It is

likely such treatments are not needed for patients with mild bronchiectasis who have few symptoms when clinically stable and have few chest infections. The largest anti-inflammatory therapies studied to date are the macrolides. In addition to antibacterial properties, macrolides are thought to have immunomodulatory and anti-inflammatory properties. Over recent years, several studies have been done assessing the role of long term macrolides in stable bronchiectasis.

Tsang *et al* performed a randomised double blind placebo controlled study showing an improvement in lung function and sputum volume reduction in patients treated with 8 weeks of 500mg b.d erythromycin (Tsang *et al*, 1999). It did not show a difference in microbial load or inflammatory markers but the majority of patients were colonised with *Pseudomonas aeruginosa*.

Wong *et al* confirmed these findings in the Effectiveness of Macrolides in patients with Bronchiectasis using Azithromycin to control Exacerbations (EMBRACE) trial (Wong *et al*, 2012). This was a randomised double-blind placebo controlled trial of 141 patients randomised to either taking azithromycin 500mg three times a week or placebo for 6 months. They showed a significant reduction in the rate of event-based exacerbation from 1.57 in the placebo group to 0.59 with the azithromycin group. There was no statistically significant difference in lung function or quality of life. Macrolide resistance testing was not routinely undertaken in this study but 4% in the azithromycin group had macrolide resistant *Streptococcus pneumoniae* at 6 months.

The Bronchiectasis and long term Azithromycin Treatment (BAT) study in 83 bronchiectasis patients with 3 or more exacerbations in the past year were randomised to either 250mg azithromycin once daily or placebo for 1 year (Altenburg *et al*, 2013). Results showed a significant decrease in exacerbation frequency in the azithromycin group with a longer time to first exacerbation during treatment. FEV_1 and FVC improved with azithromycin, as did quality of life as assessed by SGRQ. Sputum microbiology was similar at baseline and at 1 year but macrolide resistance was recorded in 88% of the isolates tested (53 of 60 pathogens tested in 20patients in the azithromycin arm) compared with 26% (29 of 112

pathogens tested from 22 patients) in the placebo group during treatment. Adverse reactions were reported, mainly gastrointestinal symptoms, but these were not severe enough to discontinue treatment.

The Bronchiectasis and Low dose Erythromycin Study (BLESS) investigated the effect on exacerbations rates and resistance rates post 1-year therapy with low dose erythromycin (Serisier *et al*, 2013). Patients were randomised to low dose erythromycin or placebo for 48 weeks. Results showed a significant reduction in exacerbation frequency, reduced FEV_1 decline but an increase in macrolide resistance. Erythromycin was well tolerated with no significant adverse effects. In the literature, there is evidence of increased risk of cardiac death due to prolonged QTc and arrthymias with the use of macrolides (Ray *et al*, 2012). No difference in QTc values or arrthymias was reported at this low dose, in the BLESS trial. Erythromycin increased the proportion of macrolide resistant oropharyngeal streptococci.

There is debate whether macrolides are anti-infective, anti-inflammatory or both. All three studies showed in comparison to placebo, macrolide therapy reduced exacerbations (Wong *et al*, 2012; Altenburg *et al*, 2013 & Serisier *et al*, 2013). It is clear that it was not dependent on the macrolide chosen or the frequency of delivery. Reducing exacerbations is key to improving the morbidity of bronchiectasis and these studies support the long-term use of macrolides. There are however concerns with macrolides concerning their toxicity in a middle age and elderly cohort. The concerns are the risk of non-tuberculous mycobacteria, cardiovascular mortality and pneumococcal resistance to macrolides (Adjemian *et al*, 2012 & Olivier *et al*, 2003). In bronchiectasis, macrolide resistance in this group would make the treatment of non-tuberculous mycobacteria very difficult to treat. This is of major concern because non-tuberculous mycobacteria are isolated in 5-10% of cases in Europe but 30-40% cases in the US (Adjemian *et al*, 2012 & Olivier *et al*, 2003). Recent reviews have shown that macrolide use can increase cardiovascular mortality, which would be concern to a middle and elderly aged cohort (Ray *et al*, 2012 & Schembri *et al*, 2013). It has been shown in these studies that there is a significant increase in

pneumococcal resistance to macrolides (Altenburg *et al*, 2013 & Serisier *et al*, 2013). It is not known however whether this has any clinical significance. In the studies to date up to one year, these have not been of any clinical significance.

1.13 Stepwise management of bronchiectasis

Exacerbations should be treated with antibiotics based on previous sputum culture. If patients are experiencing 3 or more exacerbations in a year, which is considered to be a marker of disease severity then the above stable treatments should be considered. The updated BTS guidelines have suggested a stepwise management plan as demonstrated in figure 8.

Figure 8.

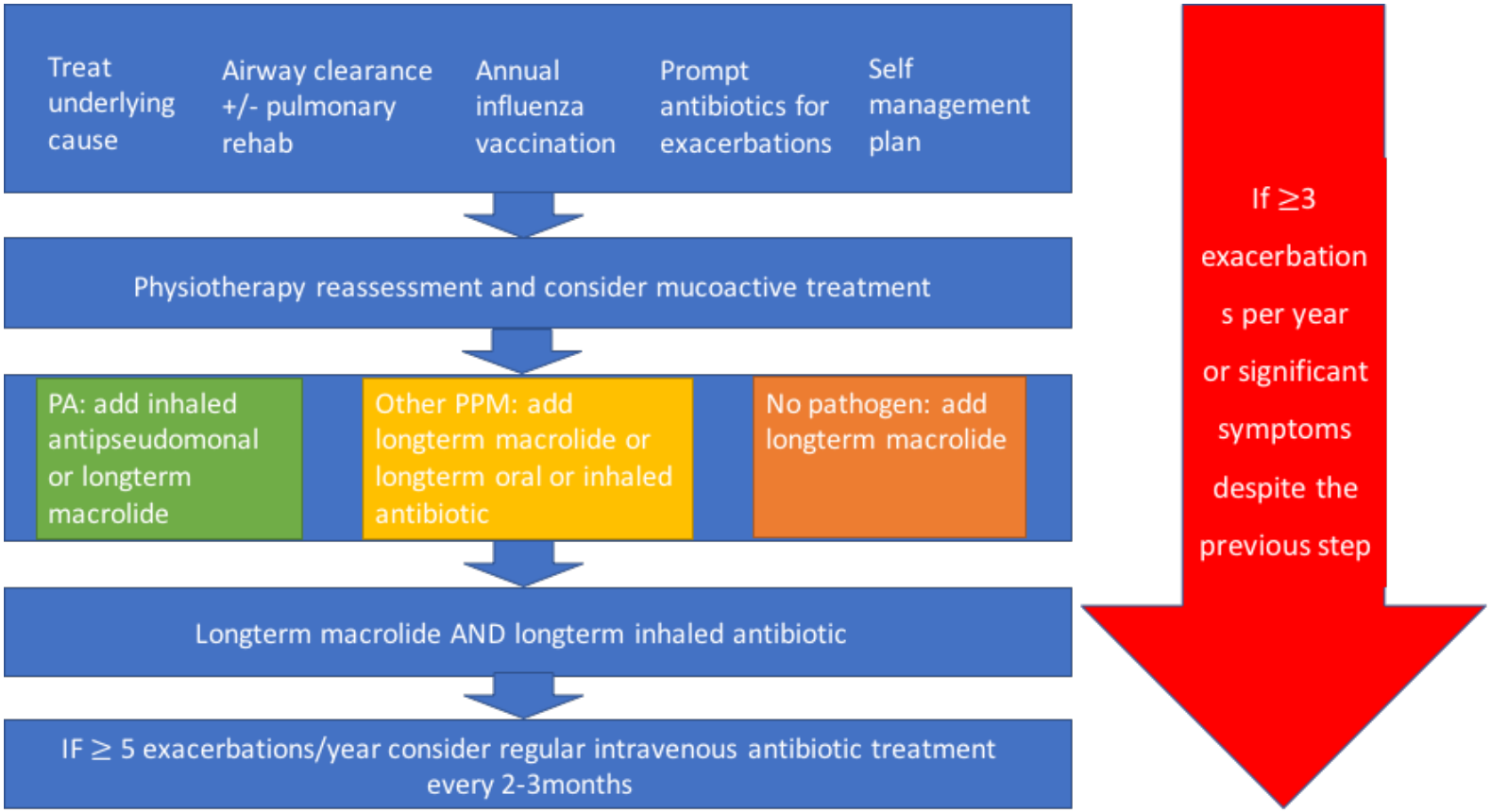


Figure 8. The stepwise management recommended in patients with 3 or more exacerbations per year. PA: *Pseudomonas aeruginosa*, PPM: potentially pathogenic microorganisms.

1.14 Monitoring of disease

Antibiotics and chest physiotherapy are the accepted forms of treatment for exacerbations of bronchiectasis but there are no randomised placebo-controlled trials examining the efficacy of treatment. Validated endpoints to assess response to treatment has been investigated by Murray and colleagues. They investigated several endpoints (24hr sputum volume, forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), incremental shuttle walk test, qualitative sputum microbiology, white cell count, erythrocyte sedimentation rate, C-reactive protein (CRP) and St George's Respiratory Questionnaire (SGRQ)) after 14days of intravenous antibiotics for patients colonised with *P. aeruginosa* and other potentially pathogenic microorganisms. They found all clinical markers improved with treatment irrespective of pathogenic organism except for FEV_1 and FVC. They reported the most responsive markers to include 24hr sputum volume (reduced by >50% in 80% of all cases), sputum bacterial clearance (in 78.1% of all cases), CRP with more than 75% reduction in 62.5% of all cases and health related quality of life score as measured by the St George's Respiratory Questionnaire (SGRQ) improved by the minimum clinically important difference or more (4 or more units) in 89.7% of all cases. They found 24hr sputum volume, CRP and SGRQ scores all improved independent of bacterial clearance (Murray *et al*, 2009c).

Phase 3 research studies (Haworth *et al*, 2014; Wong *et al*, 2012; Serisier *et al*, 2013) are using time to next exacerbation or exacerbation frequency as primary endpoints but these have not been validated endpoints for use in bronchiectasis. Several questionnaires assessing quality of life have been validated for use as endpoints in bronchiectasis including The Leicester Cough Questionnaire, The St George's Respiratory Questionnaire, Quality of Life-Bronchiectasis and Short form-36. A meta-analysis of 38 studies was performed by Spinou and colleagues to assess their psychometric properties and the strength of their associations with other clinical endpoints. They reported good test-retest ability and moderate to good internal consistency. The authors reported health-related quality of life scores correlated better with subjective clinical endpoints such as degree of dyspnoea and

fatigue then objective measures of exercise capacity, FEV_1 and radiological extent of bronchiectasis on CT scan ($P<0.0001$ for all endpoints) (Spinou *et al*, 2016).

There are no studies in bronchiectasis comparing different monitoring schedules in stable disease of bronchiectasis or correlated strategies to outcomes. Factors associated with increased risk of exacerbations, hospitalisations and mortality in bronchiectasis include age, body mass index, past history of exacerbations and or hospitalisations, persistent infection, especially with *P. aeruginosa*, dyspnoea as measured on the MRC scale, radiological extent of disease and FEV_1 and so it would be understandable to monitor these variables in addition to quality of life as these tools may assess components of health status in bronchiectasis not measured using other outcomes (Spinou *et al*, 2016). The time intervals of monitoring are not known but all patients with bronchiectasis should undergo routine monitoring tailored to their disease severity in order to identify disease progression, pathogen emergence and modify treatment where necessary (Hill *et al*, 2018).

These variables have recently been incorporated into two severity scoring systems – the Bronchiectasis Severity Index (BSI) (Chalmers *et al*, 2013) and the FACED score (Martinez-Garcia *et al*, 2014). These scoring systems appear to be comparable but the BSI has higher sensitivity for predicting those at further risk of exacerbation (McDonnell *et al*, 2016). Studies have also suggested that the existence of comorbidity can increase severity and therefore the bronchiectasis aetiology and comorbidity index (BACI) was produced and whilst it appears to have a higher predictive power for 5year mortality when used in conjunction with the BSI score, its role in the monitoring of disease is yet unknown (McDonnell *et al*, 2016b).

The new bronchiectasis guideline group (Hill *et al,* 2018) recommend certain criteria necessitating specialist input in secondary care for monitoring of patients. These include severity markers of 3 or more exacerbations/year, colonisation with *P. aeruginosa*, non-tuberculous mycobacteria or methicillin-resistant *S. aureus* colonisation (MRSA), deteriorating lung function, patients receiving any method of long-term antibiotic delivery and patients with diagnoses of rheumatoid arthritis,

immune deficiency, inflammatory bowel disease, primary ciliary dyskinesia, ABPA or patients with advanced disease and considering transplantation.

1.15 Aims

There were three main aims for this thesis.

1) To phenotype stable patients with bronchiectasis.
2) To investigate the importance of sputum bacterial load on outpatient exacerbations of bronchiectasis.
3) To evaluate validated clinical endpoints in bronchiectasis.

To investigate the first aim patients with bronchiectasis were recruited when clinically stable. Currently disease severity is assessed according to the Bronchiectasis Severity Index (BSI) score. This study investigated different clinical and laboratory markers to see if they correlated with the bronchiectasis severity score to see if disease severity can be further explored with markers not included in the original BSI score.

The second aim was investigated by designing a study where patients with bronchiectasis were assessed when clinically stable, at start of exacerbation and after 14 days of oral antibiotic therapy. Clinical, bacteriological and inflammatory markers were all assessed at each time point including quantitative microbiology to assess the importance of a rise in bacterial load has on outpatient exacerbations of bronchiectasis.

There is a lack of validated clinical endpoints in bronchiectasis. Different endpoints have been validated for use in bronchiectasis but the none of these assess functional capacity. There are no randomised controlled trials assessing the different clinical endpoints. In order to investigate this third aim, a study was designed to validate the incremental shuttle walk test for use in bronchiectasis.

Chapter 2:
Material and Methods

2.0 Material and methods

2.1 Ethical approval

Ethical approval was granted by the West of Scotland Research ethics service, REC reference 13/WS/0230. All patients provided informed written consent and were recruited from a single centre.

2.2 Patient recruitment

All patients aged 18years and older with a radiologically confirmed diagnosis of bronchiectasis were eligible for inclusion in the separate studies. For all the studies reported in this thesis patients with clinical and radiological bronchiectasis were included but those with a primary diagnosis of chronic obstructive pulmonary disease, poorly controlled asthma, active sarcoidosis, active lung malignancy or active pulmonary mycobacterial disease were excluded.

A clinical diagnosis of bronchiectasis included patients with a history of chronic cough with daily sputum production who had previously experienced exacerbations or bronchiectasis requiring antibiotics. All patients attended a dedicated bronchiectasis clinic in a tertiary centre – The Royal Infirmary of Edinburgh.

A radiological diagnosis of bronchiectasis was defined as a high resolution computer tomography scan preformed within the last 5years which was reported by a trained radiologist and demonstrated bronchial dilatation, defined as a wider luminal diameter of a bronchus in respect to the neighbouring bronchial artery.

Patients were required to be clinically stable with no exacerbations requiring antibiotics or oral corticosteroid prescription and no changes to regular respiratory medications in the preceding 6 weeks. For exacerbations, patients phoned the clinical research fellow when they felt they were having an exacerbation and if the fellow thought antibiotic treatment was warranted then they were reviewed and entered into the study. When patients were recalled due to an exacerbation, only the first exacerbation after the clinically stable visit was included.

2.3 Sputum

2.3.1 Sputum volume

Patients were asked to provide two sputum samples whenever attending a clinical visit. The first was a 'Spontaneous sputum' sample which was all sputum expectorated within the first 4 hours of rising from bed on the day of the visit. Sputum was collected in a sterile, transparent container. The second sample was a '24-hour sputum' collection which included all the sputum expectorated for the full 24hour period the day before the clinical visit. Again, sputum was collected in a sterile transparent container.

2.3.2 Sputum colour

All sputum samples were graded for sputum colour using a validated sputum colour chart (Murray *et al*, 2009). Mucoid sputum was graded a 1, mucopurulent sputum was graded a 2 and purulent sputum was graded a 3 or 4 depending on the colour with increasing purulence awarded a higher number (Figure 1).

Figure 1.

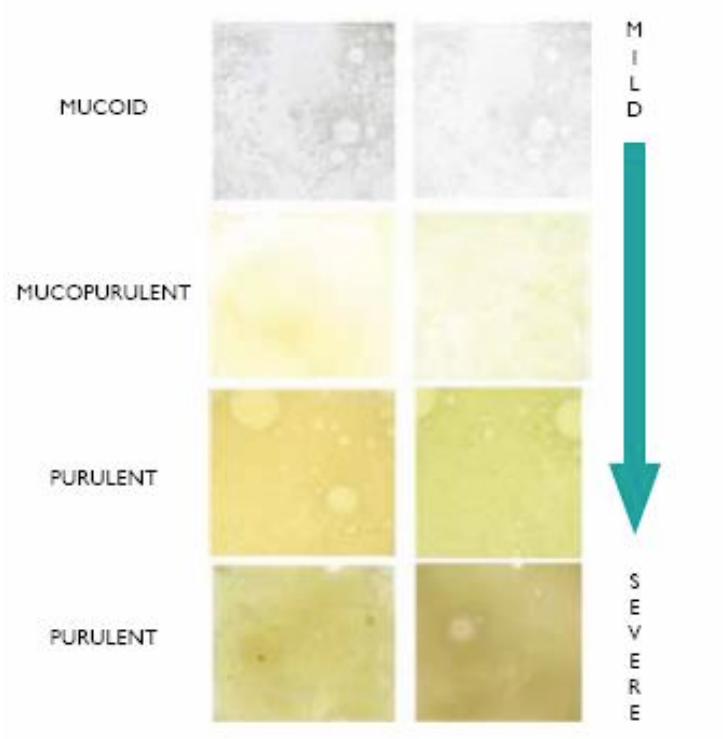


Figure 1. Sputum colour chart (Murray *et al,* 2009)

2.4 Bacteriology

2.4.1 Quantitative bacteriology

Sputum samples were processed as quickly as possible after receipt. Sputum was prepared by adding an equal volume of 0.1% dithiothreitol to at least 1millilitre of fresh sputum. Samples were vigorously vortexed to produce a liquefied and homogenised sample. The sample was then serially diluted with 0.85 % saline to achieve dilutional factors of 10^{-1} and 10^{-4}. 100microlitres aliquots of diluted sputum of concentrations $x10^{-2}$ and $x10^{-4}$ were then inoculated across the surface of different culture media and spread using a sterile hockey stick spreader (Pye *et al*, 1995). Three different culture plates were used at each of the two dilutions: Pseudomonas isolation agar (Difco) which is selective for *Pseudomonal* species, chocolate blood agar with bacitracin (Oxoid) and horse blood agar (Oxoid). The plates were incubated for 48 hours at $37^{o}C$ in an aerobic incubator except for the chocolate blood agar plates which were incubated at $37^{o}C$ for 48 hours in a 5% carbon dioxide enriched incubator. Colony forming units for each different pathogen identified were counted at 48 hours and the bacterial count was calculated as follows: Number of colony forming units x 2 x 10 x dilutional factor to give the number of colony forming units per millilitre (CFU/ml).

The remainder of the fresh sputum was ultra-centrifuged at 30000g for 90 minutes at $4^{o}C$ to produce a pellet and supernatant (Hill *et al*, 1999). The soluble portion was aspirated into small aliquots and stored at $-70^{o}C$ until it was defrosted thoroughly at room temperature for further analysis.

2.4.2 Qualitative bacteriology

Pathogens were identified using Gram stain and specific standardised identification tests for the more commonly identified pathogens are outlined below.

2.4.2.1 Gram stain

Ten microliters of fresh sputum was smeared across a glass slide using a sterile 10ml sterile loop. An equal volume of sterile saline was added to the slide and the sputum was spread thinly across the slide. The slide was air dried and heat fixed by

passing through an open flame until the moisture had evaporated. Methylviolet was added and left for one minute before rinsing the slide with deionised water. Iodine was then added and left for a further minute before rinsing with deionised water again. Acetone was subsequently added as a decolourising agent followed by rinsing with deionised water. A counter stain of basic fuschin was then used to flood the slide for one minute before rinsing with deionised water. The slide was air dried before observing under oil immersion at low (x100) and high (x1000) magnification. The bacterial cell colour was described as pink (gram negative) or purple (gram positive) and the morphology (rods or cones) was noted to help identification.

2.4.2.2 *Haemophilus influenzae*

These colonies appear small, round and convex on chocolate blood agar plates which have been incubated at 37^{o}C with 5% carbon dioxide, usually visible after 24hours (figure 2a). Gram staining shows gram negative spherical, oval or rod-like cells of less than 1μm diameter. Further identification of *H. influenzae* was carried out using X and V factors, which also helped distinguish them from *H. parainfluenzae*. One colony was gently lifted with a sterile loop and emulsified in distilled water to produce a light suspension. 100μl of this suspension was then inoculated onto nutrient agar and spread evenly with a sterile hockey stick spreader. Three filter paper discs 'X' – containing protoporphyrin IX and haemin, 'V' – containing nicotinamide adenine dinucleotide and 'XV' –containing both the above, were placed on the agar plate at equidistant from each other with a minimum of 3.5cm between the discs (Figure 2b). Plates were incubated for 24 hours at 37^{o}C and examined for growth around each disc as per table 1.

Table 1.

Factor	*Haemophilus influenzae*	*Haemophilus parainfluenzae*
X	Negative	Negative
V	Negative	Positive
XV	Positive	Negative

Table 1. Test results for growth around the respective discs for *H. influenzae* and *H. parainfluenzae.*

Figure 2a.

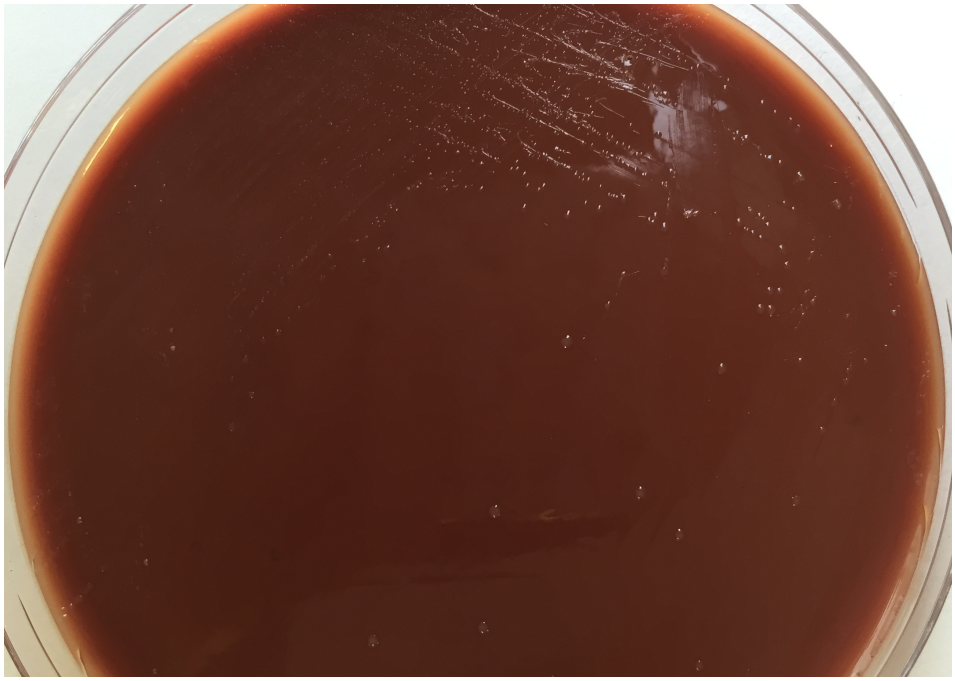


Figure 2b.

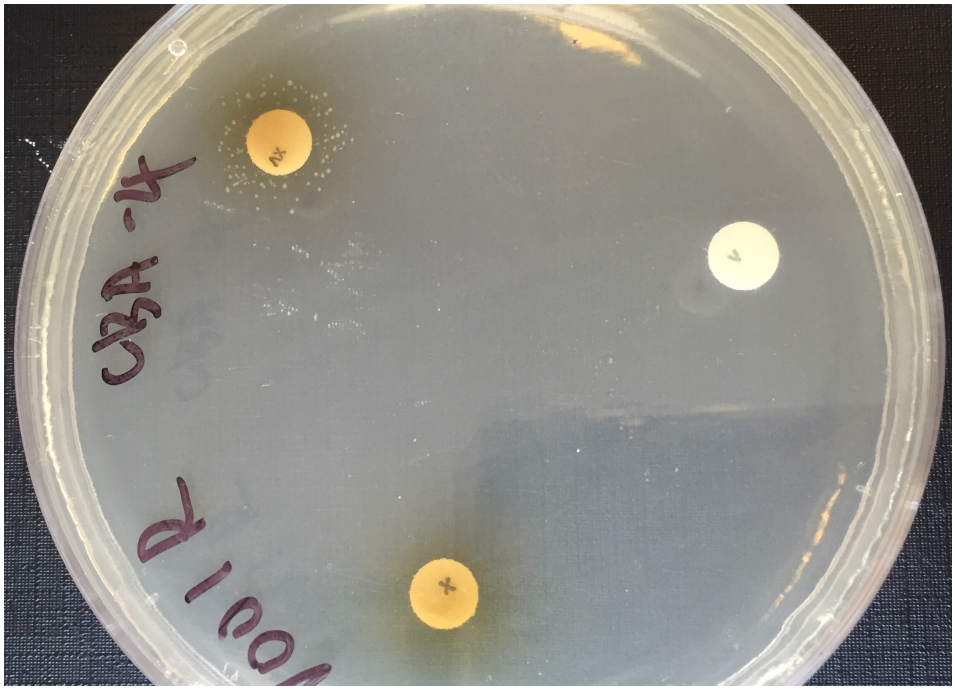


Figure 2. *Haemophilus influenzae*, a) classical appearance of colonies on chocolate blood agar, b) positive XV identification test.

2.4.2.3 *Pseudomonas aeruginosa*

Morphological identification of Pseudomonas aeruginosa included several

characteristics. The colony appearance varied but the most common type is a large, oval convex shape with a rough appearance on primarily *Pseudomonas* isolation agar plates (figure 3a) but in mucoid strains the colonies coalesce and appear gel like (figure 3b). Colonies may have a greeny-blue appearance due to production of pyocyanin or yellow due to the production of pyoverdin. *Pseudomonal* species can have a characteristic smell of aminoacetophenone. Not all strains produce the pigment pyocyanin and hence further identification can be carried out using the commercially available API20NE kits (biomerieux UK limited, Basingstoke, Hampshire) according to manufacturer's instructions. This includes the sterile emulsification of potential colonies with 2mls of 0.85% sterile saline. The suspension (approximately 0.5McFarland standard) is then added to dehydrated substances present on the API20NE strip of microtubules (figure 3c). Strips are incubated for 24 hours before the addition to reagents an interpretation of reactions (as per manufacturer's instructions) prior to potential further incubation for 24 hours. The resultant biochemical reaction profile is converted into an octal profile number and decoded using the Analytical Profile Index (API database Vn6.0, APILAB software Vn3.3.3, Apilab Plus; biomerieux).

Figure 3a.

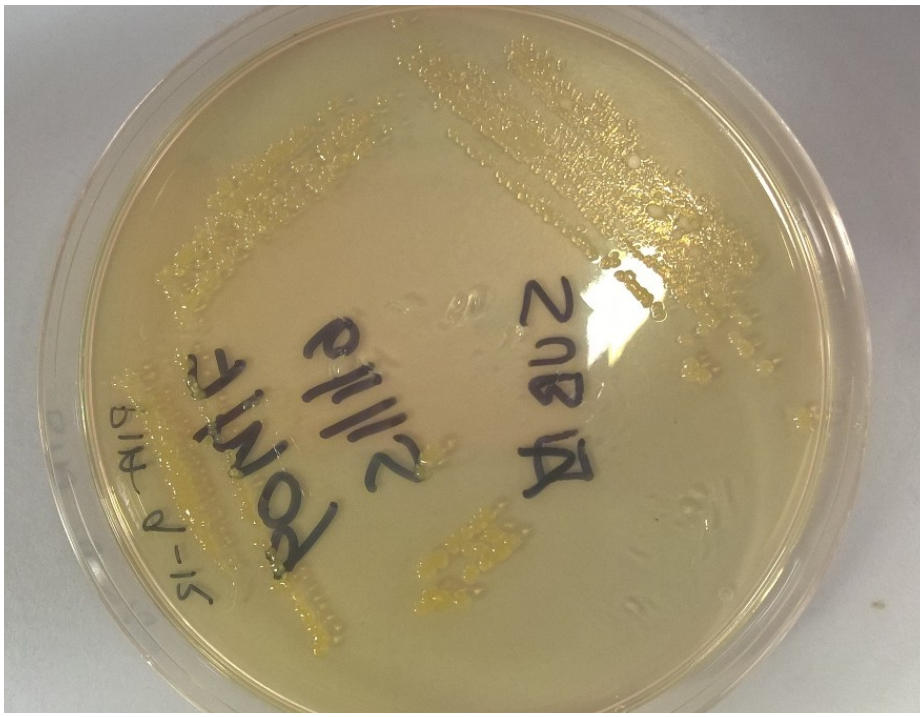


Figure 3b.

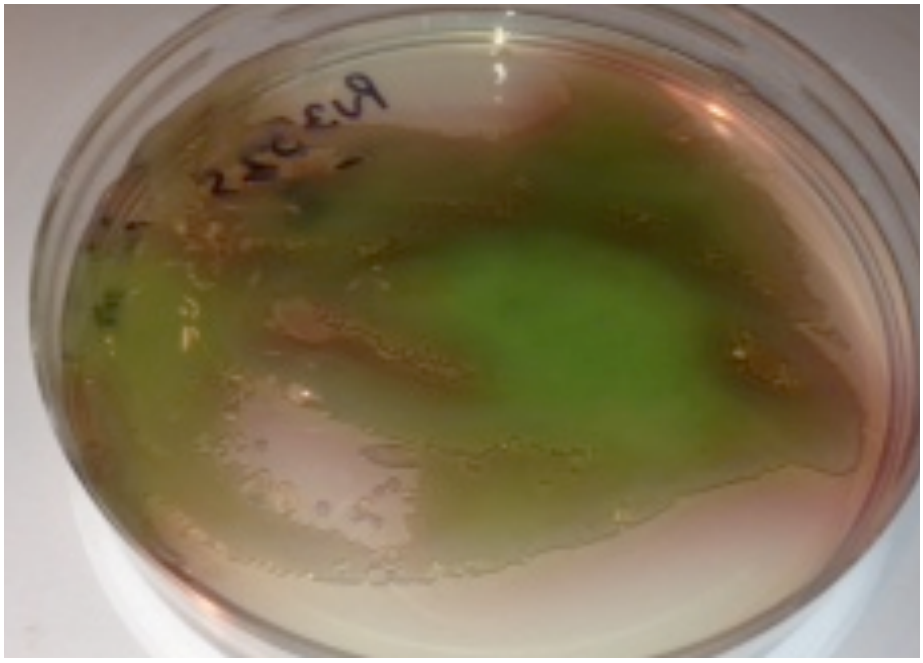


Figure 3c.

Figure 3. *Pseudomonas aeruginosa*; a) classic colonies on *Pseudomonas* isolation plate, b)mucoid *Pseudomonas* isolated in the lab, c)API20NE commercial test kit.

2.4.2.4 *Streptococcus pneumoniae*

Streptococcus pneumoniae colonies are primarily grown on horse blood agar and appear white, 1-2mm in diameter with a depressed centre and raised edges giving them the classic draughtsman appearance with underlying haemolysis (figure 4a). They appear positive on Gram staining and morphologically spherical, either in pairs, chains or clusters. Further identification is carried out but assessing the sensitivity of the colony to ethylhydrocupreine hydrochloride (optochin test). The ethylhydrocupreine hydrochloride containing optochin disc causes lysis of *Streptococcus pneumoniae* and so is placed on a horse blood agar plate that has been freshly inoculated with *Streptococcus pneumoniae* colonies using a sterile wire loop

to gently lift the colonies and streak across the plate. The plate is incubated for 24 hours at 37^{o}C in enriched 5% carbon dioxide conditions. A positive result was defined as ≥ 5mm radius of growth inhibition around the optochin disc (Figure 4b).

Figure 4a.

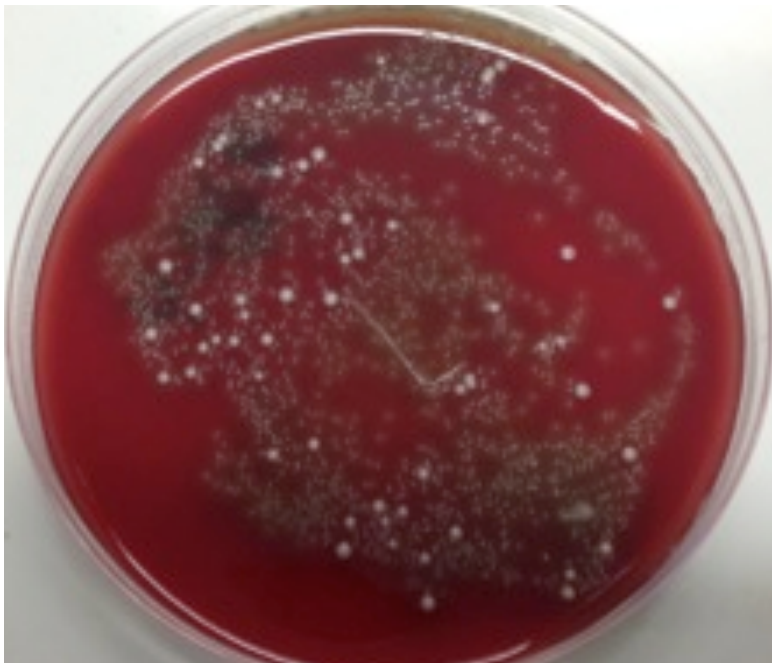


Figure 4b.

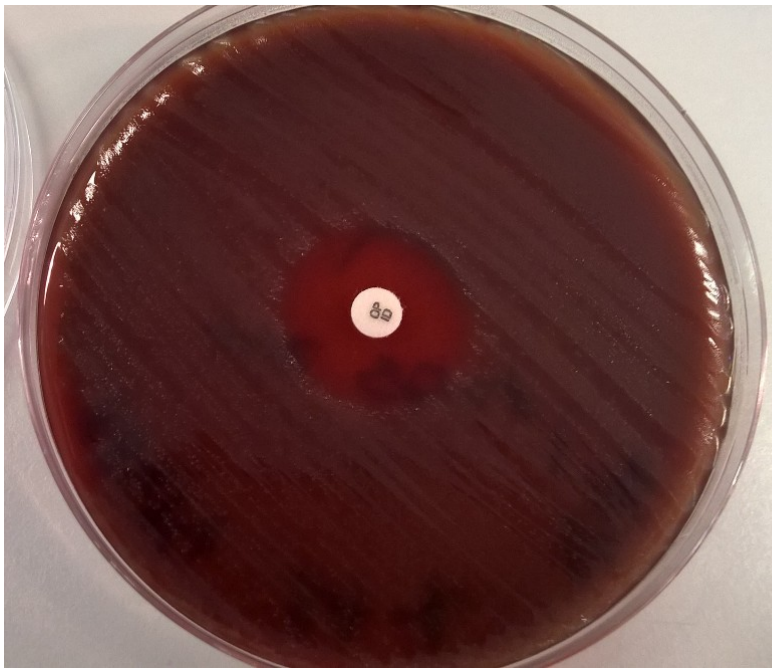


Figure 4. *Streptococcus pneumoniae*; a) classic appearance of Streptococcus pneumoniae, b) positive optochin test

2.4.2.5 *Moraxella catarrhalis*

The primary isolation media used to Moraxella species is horse blood agar, on which their appearance is grey, white or yellowy opaque colonies that are 1-3mm big and convex in shape (figure 5a). On chocolate blood agar, they can appear pinkish brown and difficult to distinguish from *Neisseria gonorrhoea* (figure 5b). They stain negative on Gram staining and appear as cocci, approximately 0.6-1.0mm diameter. Further clarification on identity can sought with the positive oxidase reaction. Filter paper was soaked in the test reagent N, N, N‟, N‟-tetra-methyl-p-phenylenediamine dihydrochloride. A sterile wooden stick was used to select one colony and rub it onto the pre-soaked filter paper. A positive result was confirmed with the development of a blue colour, indicating oxidase production within ten seconds. A negative result was indicated by an absence of colour.

Figure 5a.

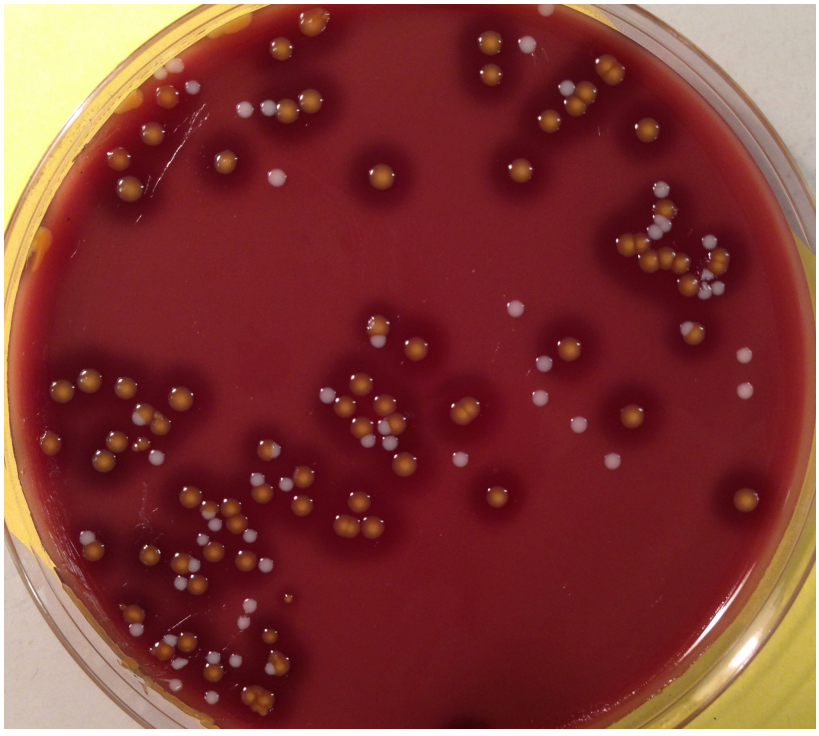


Figure 5b.

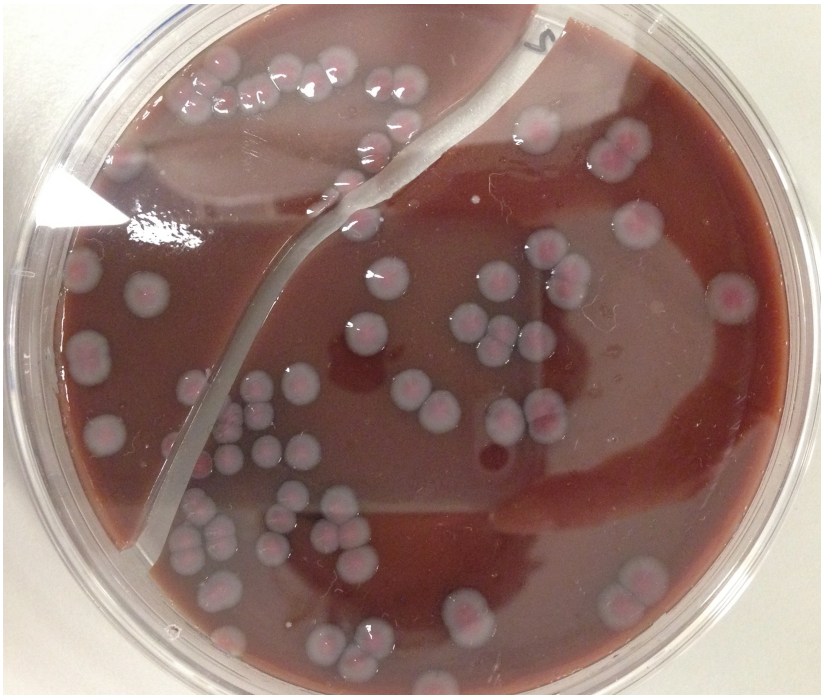


Figure 5. Moraxella catarrhalis; a) classical appearance on horse blood agar, b) pinky-brown colonies on chocolate blood agar.

2.4.2.6 *Staphylococcus aureus*

Colonies of *Staphylococcus aureus* are opaque and creamy white or yellow-orange in colour (figure 6a). They are primarily grown on horse blood agar and stain positive on Gram staining. They are cocci which can occur singularly, in pairs or in clusters. Further identification is confirmed using the commercially available Dryspot Staphytect Plus kit. This is a latex slide agglutination test where blue latex particles coated with both porcine, fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of *Staphylococcus aureus* are dried onto the test reaction area. A second test control area contains blue latex particles sensitised with non-reactive globulin and acts as a negative control. An emulsion of test colonies and sterile saline is made and the suspension added to each test area with the addition of reagents as per manufacturer's instructions. A positive test results in the agglutination and clumping of the suspension (figure 6b).

Figure 6a.

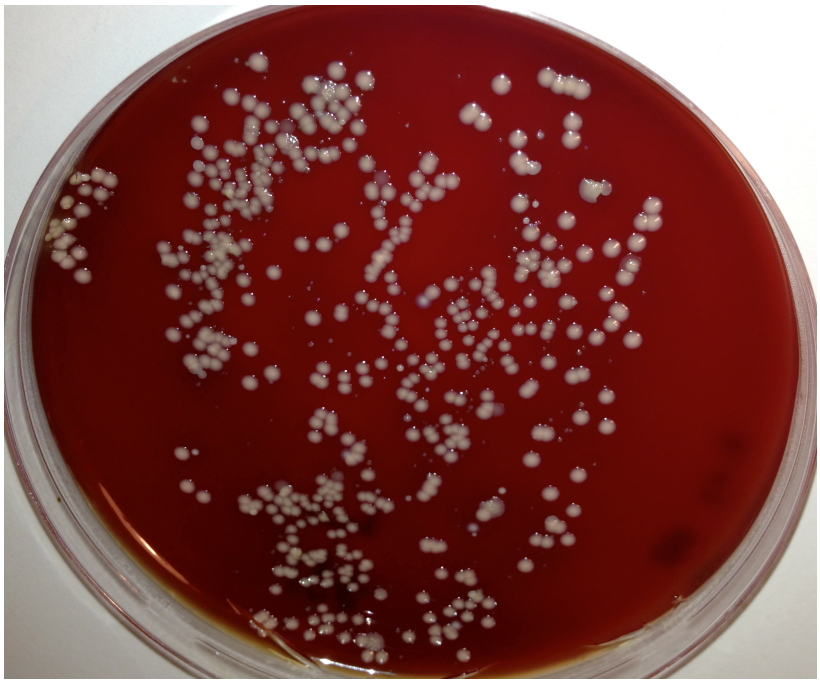


Figure 6b.

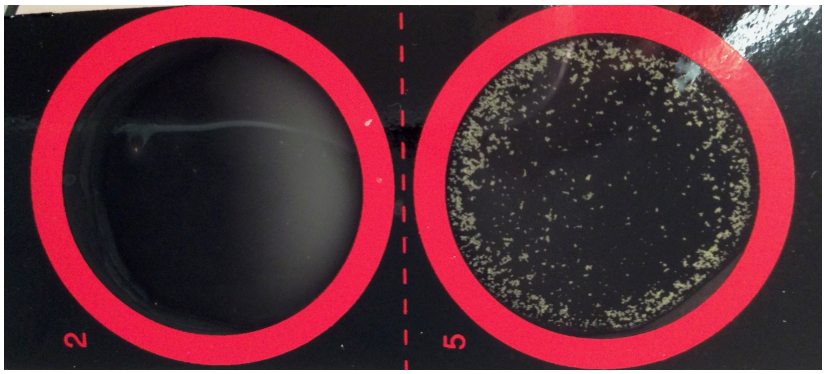


Figure 6. *Staphylococcus aureus*; a) classical appearance on horse blood agar, b) negative control test on the left and positive agglutination reaction on the right.

2.4.2.7 *Enterobacteriaceae*

These pathogens were selected from horse blood agar (figure 7a) and chocolate blood agar containing bacitracin (figure 7b). They can appear as single colonies or adopt a gel-like appearance when mucoid. The cells stain Gram negative and appear as rods on microscopy. Further identification of these pathogens is with a negative oxidase test as described above and then with the commercially available API20E (bioMerieux UK, Limited, Basingstoke, Hampshire). The preparation for this test

strip is similar to that described for the API20NE kit described above, except it is only incubated for 24 hours at 30°C (figure 7c).

Figure 7a.

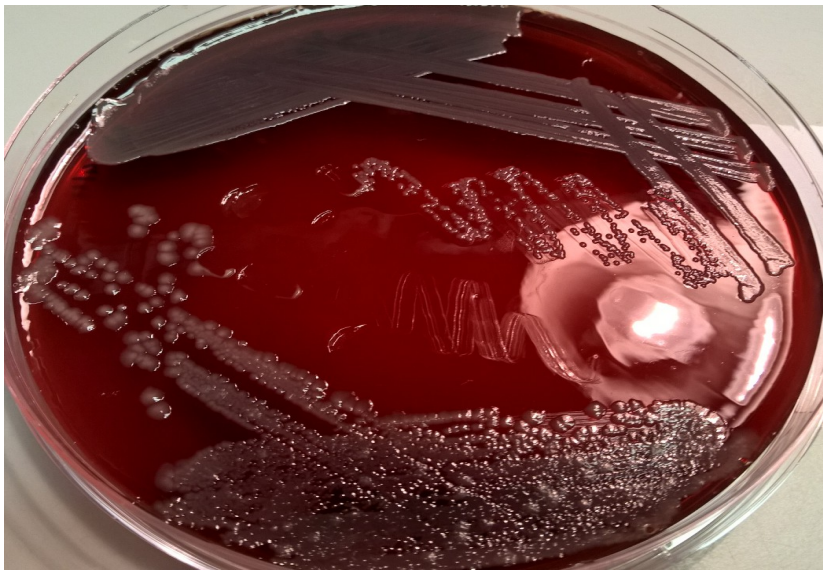


Figure 7b.

Figure 7c.

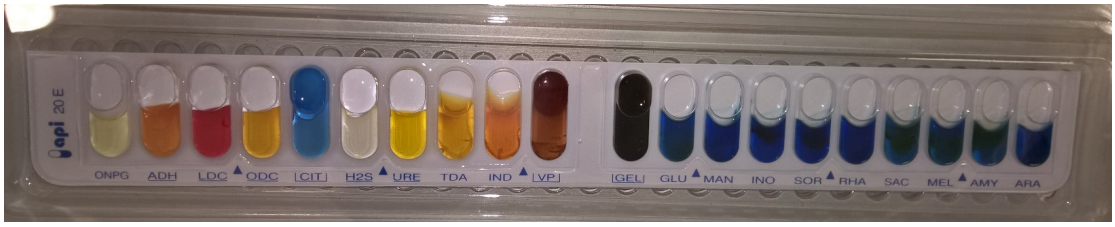


Figure 7. *Enterobacteriaceae*; a) classical appearance on horse blood agar, b) classical appearance on chocolate blood agar with bacitracin, c) API20E commercial test kit.

2.4.2.8 Mixed normal flora

There can be an abundance of mixed commensal organisms resident in the pharyngeal area and throughout the bronchial tree when no one pathogen is predominantly present. The appearance of mixed normal flora or respiratory commensals can appear on horse blood agar and chocolate blood agar containing bacitracin plates (figure 8).

Figure 8.

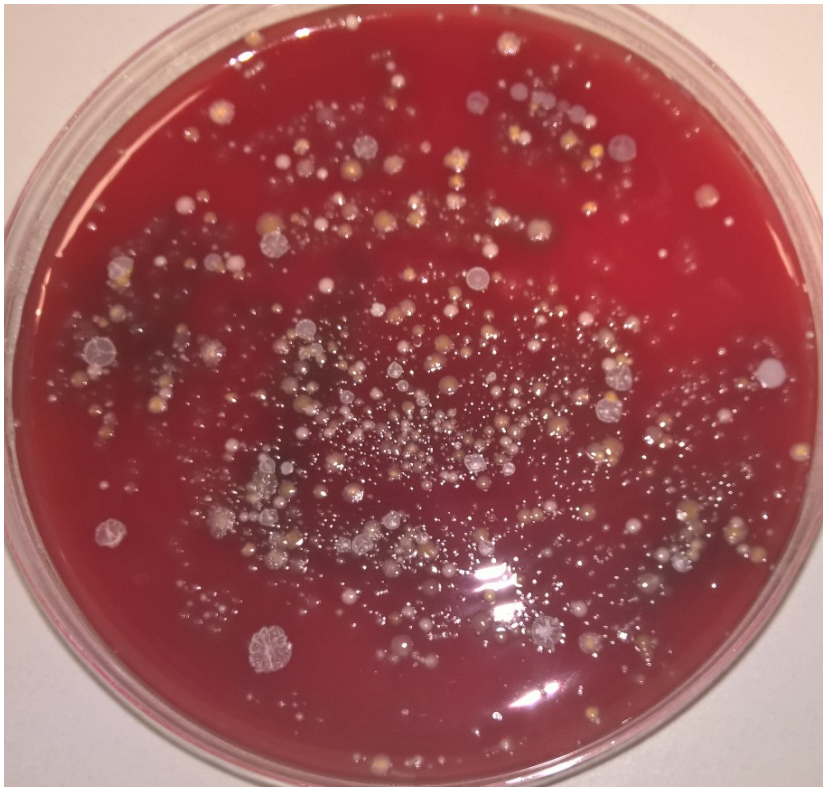


Figure 8. Mixed normal flora on horse blood agar

2.5 Sputum inflammatory marker assays

Sputum was prepared as earlier described and sputum sol frozen until required for inflammatory assays. Soluble sputum specimens were thawed at room temperature ready for use.

2.5.1 Myeloperoxidase

Myeloperoxidase (MPO) activity in the sputum was measured as a surrogate marker for the neutrophilic inflammatory response. MPO was measured from the sputum sol phase by a chromogenic substrate assay (Calbiochem). All reagents and samples

were first brought to room temperature. The test samples and standards were diluted in phosphate buffered solution as per manufacturer's instructions. 25µl of test sample or standard was added to each well of a 96 well microliter plate (Costar®). 25µl of tetramethybenzidine (TMB, Sigma) was also added to each well. The plate was incubated for 5 minutes at 25^{o}C. The reaction was stopped with the addition of 50µL of sulphuric acid to each well. The absorbance from each well was measured using a dual wavelength of 450nm and 560nm. A standard curve from the MPO standard results was formed and test sample results were interpolated from this curve and expressed as mg/ml. The MPO concentration for each standard and sample was determined in duplicate and the mean result determined for each.

2.5.2 Neutrophil elastase

Sputum neutrophil elastase was measured as a surrogate marker of the neutrophilic inflammatory response. Neutrophil elastase was measured spectrophotometrically as the rate of change of optical density. The chromogenic synthetic substrate methoxysuccinyl-ala-ala-pro-val-paranitroanilide (MeoSAAPVpNa 4-(2-hydroxyethyl)- 1-piperazineethanesulfonic acid) (Sigma) whilst not specific for neutrophil elastase can be used to detect it. Firstly, a standard curve for the rate of change in optical density by incubating the chromogen with known quantities of human neutrophil elastase is formed (Sigma Aldrich). Both the standards and test samples are diluted in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution. 40µL of standard or sample were added to a 96 well microtiter plate (Costar®). 40µl of MeoSAAPvn was added to each well and samples read immediately at 405nm, 37ºC for a minimum of thirty minutes with readings every two minutes. The rate of change in optical density is converted into elastase activity and expressed in units per milligram. The elastase concentration for each sample and standard was assessed in duplicate and the mean determined for each.

2.5.3 Interleukin-8

Interleukin-8 concentrations were assessed as a marker of neutrophil chemotaxis. The sputum sol phase from previously frozen samples stored at -70^{o}C was used and samples were first brought down to room temperature. The commercially available

specific enzyme linked immunosorbent assay (ELISA, R+D systems, Abingdon, UK) that has previously validated for use in sputum (Stockley & Bayley, 2000) was performed according to manufacturer's instructions.

2.6 Systemic inflammation

Venous blood (15mls) was collected and analysed for full blood count (FBC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), urea & electrolytes (U&Es). The normal ranges for the most pertinent markers are: white cell count 4-11 $x10^9$/L; ESR for males (age/2) mm/hr and for females (age + 10/2) mm/hr; CRP <5 mg/L, urea 2.5-6.6 mmol/L and creatinine 60-120umol/L.

5mls of blood was centrifuged at 750g for 10 minutes and the supernatant collected and stored at -70^{o}C for measuring pro and anti-inflammatory cytokines and chemoattractants by cytometric bead array (BD^{TM} Cytometric Bead Array Kits). The cytometric bead array was performed according to manufacturer's instructions and measured Human interleukin (IL) IL-12p70, TNFa, IL-10, IL-6, IL-1b and IL-8.

2.7 Quality of life assessments

2.7.1 The Leicester Cough Questionnaire (LCQ)

This 19-item questionnaire assesses 3 domains including physical (8 items), psychological (7 items) and social (4 items). The questionnaire predominantly assesses the impact of participant's cough severity on health related quality of life by assessing symptoms over the preceding 2 weeks. It has been validated for use in bronchiectasis (Murray *et al*, 2009d) and issues a score ranging from 3 to 21 with a lower score indicating greater impairment of health status due to cough. The minimum clinically important difference (MCID) is 1.3 units (Birring *et al*, 2003).

2.7.2 St George's Respiratory Questionnaire (SGRQ)

This 50-item questionnaire investigates the components of symptoms (8 items), activity (16 items) and impact (26 items) on health related quality of life. Unlike the LCQ, it assesses multiple respiratory symptoms over the preceding 4 weeks. It has been validated for use in bronchiectasis (Wilson *et al,* 1997b). The total score can range from 0 to 100 with a higher score indicating greater impairment of health

status. The minimum clinically important difference is 4 units (Wilson *et al*, 1997b).

2.8 Spirometry

Patients were asked to perform spirometry according to standardised guidelines (Miller *et al,* 2005). Forced expiratory volume in 1 second (FEV_1) was measured as the maximal expired air in 1 second after taking a maximal breath in. Forced vital capacity (FVC) was measured as the total expired volume of air with maximal effort after taking a maximal breath in. Both FEV_1 and FVC results were expressed in litres (L) and as percent of predicted values for the patient's sex, age and height. The test was conducted using the MicroMedical Microloop ML3535 device (Viasys Healthcare) and calculated FEV_1/FVC ratio and mid expiratory forced expiratory flow (FEF_{25-75}) results were also calculated from the same manoeuvres and recorded. All patients performed three manoeuvres that were within 10% of each other and the highest result was recorded. All patients had performed spirometry before as part of routine clinical practise and were aware of how to perform the test.

2.9 Incremental shuttle walk test

The ISWT was performed according to standard test procedure (Singh *et al,* 1992). The patient is asked to walk a 10-metre shuttle course with the walking speed controlled using pre-recorded audio signals. Each of the twelve incremental levels last one minute. The pre-recorded audio signals beep progressively faster so that more distance is covered at each level. The test continues until the participant is unable to complete the shuttle in the designated time (0.5metres or further from the marker at the time of the audio signal) or the patient becomes too breathless. The distance completed is recorded. Patients were instructed to walk at a steady pace aiming to turn around at each end of the course at the sound of the audio signal. All were advised to continue to walk until they felt unable to maintain the required speed without becoming unduly breathless. At the start and end all patients had their heart rate, oxygen saturations and dyspnoea score according to the modified BORG dyspnoea scale (Borg, 1982) in accordance with standardised guidelines recorded (2002). The MCID has been proposed as 35m (Lee *et al*, 2014c) but successful completion of distance walked in our study was recorded in increments of 10m.

Chapter 3:

Stable state bronchiectasis: BSI and beyond in outpatients

3.1 Severity scoring systems

Bronchiectasis is a condition with relatively little research investigating its pathophysiology and the mechanism of its natural progression compared with chronic obstructive pulmonary disease, lung cancer and asthma. Therefore, until recently there was no accepted model to grade the severity of bronchiectasis and patients were routinely classified as 'mild', 'moderate' and 'severe' but there was no officially accepted definition for this. Other respiratory conditions have robust scoring systems to help guide treatment appropriately to those most at risk and with a poorer prognosis (Lange *et al*, 2012, Chalmers *et al*, 2010 & du Bois *et al,* 2011).

There is a great need for severity scoring systems to stratify patients and allocate limited resources appropriately. There are new treatments being investigated for the management of bronchiectasis, such as long term macrolide use but these are not without their limitations; risk of microbial resistance, toxicity, healthcare costs and treatment burden (Wong *et al*, 2012, Serisier *et al*, 2013 & Altenburg *et al*, 2013). Therefore, it would be appropriate to identify those with more severe disease who might benefit most from such novel therapies.

The two new scoring systems in bronchiectasis are the Bronchiectasis Severity Index (BSI) (Chalmers *et al*, 2013) which can identify patients at risk of future hospitalisation, exacerbation and mortality. The FACED Score (Martinez-Garcia *et al*, 2014) classifies the severity of bronchiectasis according to prognosis.

The Bronchiectasis Severity Index incorporates 9 variables including; age, body mass index, FEV_1 % predicted, hospital admission in the previous year, number of exacerbations in the last year, MRC dyspnoea score, *Pseudomonas aeruginosa* colonisation, colonisation with other microorganisms and radiological severity. The BSI grades bronchiectasis with a minimum score of 0 being very mild to 26 being very severe. The scores are classified into tertiles where 0-4 is thought of as a low score, 5-8 as intermediate and $\geqq 9$ is high – Table 1.

FACED score incorporates 5 dichotomised variables including **F**EV_1 % predicted, **A**ge, Presence of chronic **C**olonization by Pseudomonas aeruginosa, radiological **E**xtension and **D**yspnoea. The overall sum of scores can range from 0 to 7 points where 0-2 points is classified mild bronchiectasis, 3-4 points is moderate bronchiectasis and overall 5-7 points is classified as severe bronchiectasis – Table 1.

Several studies comparing the two scores report that they are comparable at predicting mortality and assessing severity in regards to its prognosis (Ellis *et al*, 2016 & Minov *et al*, 2015). The FACED score is easier and less time consuming but the BSI has perhaps been more comprehensively studied to show it can predict hospital admissions as well as mortality and was found to differentiate the number of exacerbations and quality of life between the different severity classifications (McDonnell *et al*, 2016). These are all well recognised primary endpoints in bronchiectasis and important considerations for future research. Both scores have been validated, the FACED was internally validated and the BSI was externally validated.

The FACED score was later amended to construct the E-FACED score which was a 9-point severity scoring system adding in the number of severe exacerbations to its existing FACED score (Martinez-Garcia *et al*, 2017). Its predictive capacity for exacerbations was high with Area under the receiver operator curve (AUC) of 0.82 for 2 or more exacerbations in 1 year and 0.87 for 1 exacerbation in 1 year. Its predictive all cause and respiratory mortality capacity was like that of the original FACED score. The E-FACED score was externally validated in 651 patients from 6 different Latin America centers. The overall sum of the dichotomised scores can range from 0 to 9 points where 0-3 points is classified mild bronchiectasis, 4-6 points is moderate bronchiectasis and overall 7-9 points is classified as severe bronchiectasis – Table 1.

Both the BSI and E-FACED scores are clinically based. We know that patients diagnosed with bronchiectasis can have multiple comorbidities and that these comorbidities can be causative or synergistic with the presence of bronchiectasis

(Leroy *et al*, 2014). Those that can cause bronchiectasis e.g. COPD and rheumatoid arthritis are known to cause a more severe phenotype of bronchiectasis with increased mortality despite treatment of the underlying condition (Hurst *et al*, 2015, Perry *et al*, 2017). It is also known that certain comorbidities that can independently occur alongside bronchiectasis such as cardiovascular disease, mental health illness, gastro-oesophageal reflux disease and some malignancies can be major contributing factors for hospital admissions and increase the healthcare utilisation and cost burden (Joish *et al*, 2013, Gale *et al,* 2012, Lee *et al*, 2014b, Mandal *et al*, 2013b, Olveira *et al*, 2013, Chung *et al*, 2015).

Table 1.

BSI		FACED		E-FACED	
% predicted FEV_1	>80% (0) 50-80% (1) 30-49% (2) <30% (3)	% predicted FEV_1	≥50%(0) <50%(2)	% predicted FEV_1	≥50%(0) <50%(2)
Age	<50 (0) 50-69 (2) 70-79 (4) 80+ (6)	Age	≥70 (0) <70 (2)	Age	≥70 (0) <70 (2)
P. aeruginosa	Yes (3) No (0)	*P. aeruginosa*	Yes (1) No (0)	*P. aeruginosa*	Yes (1) No (0)
Radiological ≥3lobes or cystic	Yes (1) No (0)	Radiological >2lobes	Yes (1) No (0)	Radiological severity	Yes (1) No (0)
MRC dyspnoea	1-3 (0) 4 (2) 5 (3)	mMRC Dyspnoea	0-2 (0) 3-4 (1)	mMRC Dyspnoea	0-2 (0) 3-4 (1)
Hospital admissions	Yes (5) No (0)			1 hospital admission in last year	Yes (2) No (0)
No. of exacerbations	0 (0) 1-2 (0) ≥3 (2)				
Body mass Index	<18.5 (2) 18.5-25 (0) 26-29 (0) ≥30 (0)				
Other micro-organism	Yes (1) No (0)				

Table to show the individual variables for each scoring system and the points assigned to each. BSI: Bronchiectasis Severity Index. FEV_1: forced expiratory volume in 1 second, *P. aeruginosa: Pseudomonas aeruginosa*, mMRC: modified Medical Research Council

Neither prognostic score (Bronchiectasis severity index or the E-FACED score) incorporated the presence of comorbidities to assess its impact on mortality. McDonnell and colleagues constructed the Bronchiectasis Aetiology Comorbidity Index (BACI) designed to predict the 5-year mortality of comorbidity diagnoses (metastatic malignancy, COPD, inflammatory bowel disease, iron deficiency anaemia, asthma, peripheral vascular disease, haematological malignancy, cognitive

impairment, chronic liver disease, diabetes, pulmonary hypertension and ischaemic heart disease). They found the BACI predicted mortality, hospital admissions, exacerbations and health-related quality of life across all BSI risk groups (P<0.0001). The hazard ratio (95% CI) for death conferred by a one point increase in the BACI score was 1.18 (1.14 - 1.23), p<0.0001. When the BACI score was used in conjunction with the BSI, the combined model was superior overall in predicting 5-year mortality with AUC (95% CI) 0.83 (0.79 – 0.87) (McDonnell *et al*, 2016b). Depending on the relative contribution of each comorbidity to future risk patients can be stratified as low risk score:0 (Estimated 5 year mortality risk 3.5%, Estimated risk of hospitalisation for severe exacerbation 11.7% over 5 years), intermediate risk score 1-5 (Estimated 5 year mortality risk 11.7%, Estimated risk of hospitalisation for severe exacerbation 14.8% over 5 years) and high risk with a score of 6 or more (Estimated 5 year mortality risk 34.9%, Estimated risk of hospitalisation for severe exacerbation 36% over 5 years).

Bronchiectasis is known to be characterised with dilated and distorted airways. The leads to a buildup of stagnant sputum because of the resultant impaired mucociliary escalator. This sputum can become infected and a neutrophilic inflammatory response ensues. Previous studies have suggested that an increase in bacterial load can cause an increase in airways inflammation and a greater risk of exacerbations (Chalmers *et al*, 2012). The neutrophilic inflammatory response is thought to be a key component in potentiating the 'vicious circle' of lung damage in bronchiectasis (Dente *et al*, 2015) and is part of the cycle that novel treatments that reduce inflammation could be active. For example, there is recent growing interest in the use of macrolide therapy for their anti-inflammatory and not necessarily their anti-microbial properties (Wong *et al*, 2012, Serisier *et al,* 2013 & Altenburg *et al*, 2013).

Further work in 381 stable patients with bronchiectasis had sputum analysed for neutrophil elastase. The authors found neutrophil elastase levels were associated with the bronchiectasis severity index score (r=0.49, p<0.0001) but was not independently associated with mortality rates during a 3yr follow up period.

Neutrophil elastase did correlate with Medical research council dyspnoea score ($r=0.34$, $p<0.0001$), FEV_1 % predicted ($r=0.33$, $p<0.0001$), radiological extent of bronchiectasis ($r=0.29$, $p<0.0001$). Within the 3yr follow up period elevated neutrophil elastase levels were found to associate with higher rates of exacerbation and decline in FEV_1 ($P<0.0001$). The authors found neutrophil elastase to rise in exacerbations and have good discrimination for severe exacerbations and all-cause mortality (Chalmers et al, 2017).

Elevation of systemic inflammatory markers such as CRP, white cell count and Erythrocyte sedimentation rate have been found to correlate with disease severity and lung function in clinically stable patients with bronchiectasis (Wilson et al, 1998). Further work by Hsieh and colleagues investigated the role of high sensitivity CRP in stable patients and found it significantly correlated with high resolution computer tomography (HRCT) severity scores of bronchiectasis as assessed by Brody and colleagues and with oxygen saturation levels.

The Bronchiectasis Severity Index has been demonstrated to be able to predict mortality, hospitalization, future exacerbations and quality of life in stable patients with bronchiectasis based on several clinical parameters. The ongoing inflammatory response present in patients with bronchiectasis, perhaps because of chronic infection and the importance of the neutrophilic inflammatory response in its pathogenesis might indicate that sputum and serum inflammatory markers may be additive to the clinical parameters in assessing severity and predicting mortality.

The BSI incorporates 9 clinical parameters in its severity score; age, body mass index, FEV_1 % predicted, hospital admission in the previous year, number of exacerbations in the last year, MRC dyspnoea score, *Pseudomonas aeruginosa* colonisation, colonisation with other microorganisms and radiological severity. Other clinical parameters of sputum colour, quantitative bacteriology and distance walked in incremental shuttle walk test (ISWT) are also important when assessing severity of bronchiectasis.

Murray and colleagues demonstrated that sputum colour predicted bacterial colonisation (Murray *et al*, 2009) and Chalmers and colleagues demonstrated that airways bacterial load was associated with airway inflammation. They also noted that in stable patients there was a direct relationship between airway bacterial load and risk of subsequent exacerbations and severe exacerbations.

In previous work the ISWT was found to deteriorate with exacerbations and improve with treatment. It correlated with other validated markers of severity including the St George's Respiratory Questionnaire assessment of quality of life. The ISWT was validated as a useful clinical endpoint in bronchiectasis and the minimum clinical important difference was a change of 5% (Cartlidge *et al*, 2018).

This chapter aims to investigate whether serum and sputum inflammatory markers in addition to sputum colour, distance walked in ISWT and quantitative bacteriology are important in assessing disease severity of clinically stable patients and aim to see if these clinical endpoints correlated with the Bronchiectasis Severity Index.

3.2 Methodology

3.2.1 Ethics

Ethical approval was granted by the West of Scotland Research ethics service, REC reference 13/WS/0230. All patients provided informed written consent and were recruited from a single centre.

3.2.2 Recruitment and study design

Patients that were clinically stable (no antibiotic therapy for over 6 weeks for an exacerbation prior to selection) were recruited from a dedicated outpatient bronchiectasis clinic in a tertiary hospital (Royal infirmary of Edinburgh). Patient aged 16years and over with radiologically evident bronchiectasis on High Resolution Computer Tomography (HRCT) scan were invited to join and attend a consultation where the below tests were performed. Bronchiectasis severity score (BSI) was calculated for each individual patient according to previous clinical data recorded in routine clinic appointments. All patients underwent the tests below and

results analysed for each of the three severity groups 'BSI 0-4 mild', 'BSI 5-8 moderate' and 'BSI ≥9 severe'.

3.2.3 Blood inflammatory markers

Venous blood sampling was taken to monitor full blood count, urea and electrolytes, Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

3.2.4 Sputum inflammatory markers

Whole sputum samples were also centrifuged to produce supernatants on which myeloperoxidase (MPO), neutrophil elastase (NE) and interleukin – 8 (IL-8) were tested. MPO (Steinman *et al,* 1972) was assayed using a chromogenic substrate assay (Stockley et al, 2000), free neutrophil elastase (NE) (Buttle *et al*, 1990) was measured by spectrophotometry with a synthetic substrate (methoxysuccinyl-Ala-Ala-Pro-Val paranitroanilide; Sigma, Gillingham, UK) were tested as per standard protocol (Hill *et al*, 1999, Hill *et al*, 2000) and interleukin – 8 (IL-8) assayed using commercially available specific ELISAs (R&D Systems, Oxford, UK).

3.2.5 Sputum

Sputum colour was graded according to a visual colour chart rating. Mucoid sputum was rated 1, mucopurulent rated as 2 and purulent sputum rated as 3 or 4 depending on colour (Murray *et al*, 2009]). 24hour sputum volume was recorded by asking patients to expectorate solely into a universal container for 24hours. Qualitative and quantitative microbiology was performed on all samples.

3.2.6 Incremental shuttle walk test

The test was performed according to standard test procedure [Singh *et al*, 1992] and consisted of a 10-metre shuttle course on a flat surface with the walking speed controlled using pre-recorded audio signals. The test continues until the participant is unable to complete the shuttle in the designated time or the patient decides to stop due to symptoms (e.g. breathless, leg fatigue). The distance completed within the timeframe was recorded. The successful completion of distance walked in our study was recorded in increments of 10m.

3.2.7 Qualitative and quantitative microbiology (Murray *et al,* 2011)

For analysis culture results were grouped as either positive for '*Pseudomonas aeruginosa*', 'potentially pathogenic microorganisms' (*Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus*) or 'other gram negatives' (*Enterobacter, Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia, Serratia marcescens, Proteus mirabilis, Acinetobacter baumannii, Achromobacter xylosoxidans, Citrobacter freundii* and *Aeromonas hydrophila*) and 'mixed normal flora' (no dominant pathogen cultured or presence of respiratory commensal flora only).

3.2.8 Lung function

We measured pre-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and ratio FEV_1:FVC by spirometry according to standardized guidelines [ATS guidelines]. Patients were asked to take three attempts and the best results were recorded. All patients have previously performed spirometry as part of routine clinical practise.

3.2.9 Quality of Life

Health related quality of life assessments were assessed by the St George's Respiratory Questionnaire. Quality of life was also assessed by evaluating the impact of cough by completing The Leicester Cough Questionnaire.

3.2.10 Statistical analysis

All data were analysed using Graphpad prism version 5.0a (Graphpad software, San Diego, CA, USA). For demographic and clinical variables, data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables unless specified otherwise. A P value <0.05 was considered statistically significant for each analysis. Comparison of changes between groups was performed by Analysis of Variance (ANOVA) statistical test or by using the Kruskal-Wallis test if data was not normally distributed.

3.3 Results

3.3.1 Cohort

229 patients signed consent forms to take part in the study. Patients were over recruited to compensate for dropouts. 208 patients were contactable and proceeded to a baseline visit. Of the remaining 21 patients 13 were either not contactable again to arrange a visit or there was not a suitable time for baseline visit due to ill health or recent exacerbation. 1 patient died before attending baseline visit and 7 patients changed their mind about taking part.

1 of the 208 patients that completed a baseline visit dropped out during the study and so is not included in the results analysis. Baseline characteristics are displayed below in table 2. The relatively small number of patients on long term antibiotic therapy is a reflection of local departmental practice and not of cohort severity. Of the 207 patients that continued with the study 74 had a BSI score 0-4, 79 had a BSI score 5-8 and 54 had a BSI score of 9 or above. Aetiological data is shown in table 3.

Table 2.

Characteristic	Total patients
Number	207
Age (yrs) (mean, (SD))	65.79 (10.6)
Female	110 (53)
Current smokers	9 (4)
Ex-smokers	83 (40)
Never	115 (56)
BMI Kg/m^2 (mean, (SD))	29.09 (35.54)
COPD	35 (17)
Asthma	23 (11)
Interstitial lung disease	3 (1)
Lung cancer	2 (1)
Pulm. Hypertension	1 (1)
Inactive ABPA	21 (10)
IHD	14 (7)
Other cancers	22 (11)
LTOT	2 (1)
Inhaled steroid	23 (11)
Dose (mean, (SD))	211mcg (104.4)
Oral steroid	12 (6)
Dose (mean, (SD))	7.7mg (5.3)
Nebulised bronchodilators	7 (3)
Long-term oral antibiotics	4 (2)
Long-term inhaled antibiotics	7 (3)
Cyclical IV antibiotics	1 (0.5)
Forced Expiratory volume 1s mean (SD)	2.03 (0.77)
%pred Forced Expiratory volume 1s mean (SD)	78.6 (24.0)
Bronchiectasis severity score (mean, (SD)	6.7 (4.2)
Bronchiectasis Severity Index 0-4, 5-8, ≥9	74, 79, 54

Table to show the baseline characteristics of the 207 stable patients recruited. N (%) or as otherwise stated. BMI: body mass index, COPD: chronic obstructive pulmonary disease, ABPA: allergic bronchopulmonary aspergillosis, IHD: ischaemic heart disease, IV: intravenous and LTOT: long term oxygen therapy.

Table 3.

Aetiology	Number of patients (%)
Idiopathic	92 (44)
Post infectious	55 (27)
COPD	9 (4)
Inflammatory bowel disease	7 (3)
Immunoglobulin deficiency	9 (4)
Allergic bronchopulmonary aspergillosis	13 (6)
Connective tissue disease	7 (3)
Aspiration or inhalation or GORD	15 (7)

Table to show the N (%) of patients with different aetiologies of bronchiectasis. COPD: chronic obstructive pulmonary disease, GORD: gastric oesophageal reflux disease.

3.3.2 Serum inflammatory markers results

There was a significant association between increasing BSI severity group and white cell count (WCC), neutrophil count, Erythrocyte sedimentation rate and C-reactive protein whilst clinically stable. The median $x10^9$/L (IQR) WCC for groups 0-4, 5-8 and ≥9 was 6.3 (5.3-7.9), 6.9 (5.5-8.7) and 7.9 (6-9.95), p=0.0084. The median $x10^9$/L (IQR) for neutrophil counts for groups 0-4, 5-8 and ≥9 was 3.5 (2.6-4.8), 3.98 (3.2-6.2) and 5.2 (3.5-5.7), p=0.0013. The was also increasing ESR mm/hr levels with increasing severity; 11 (16-17), 12 (7-18) and 17 (8-28.8) respectively, p=0.0065. CRP for group 0-4 was 3mg/L (1-7); group 5-8 was 3mg/L (1-8.6) and group 9 or above was 7mg/L (2.5-22.5), p=0.0045 – see Figure 1.

Figure 1.

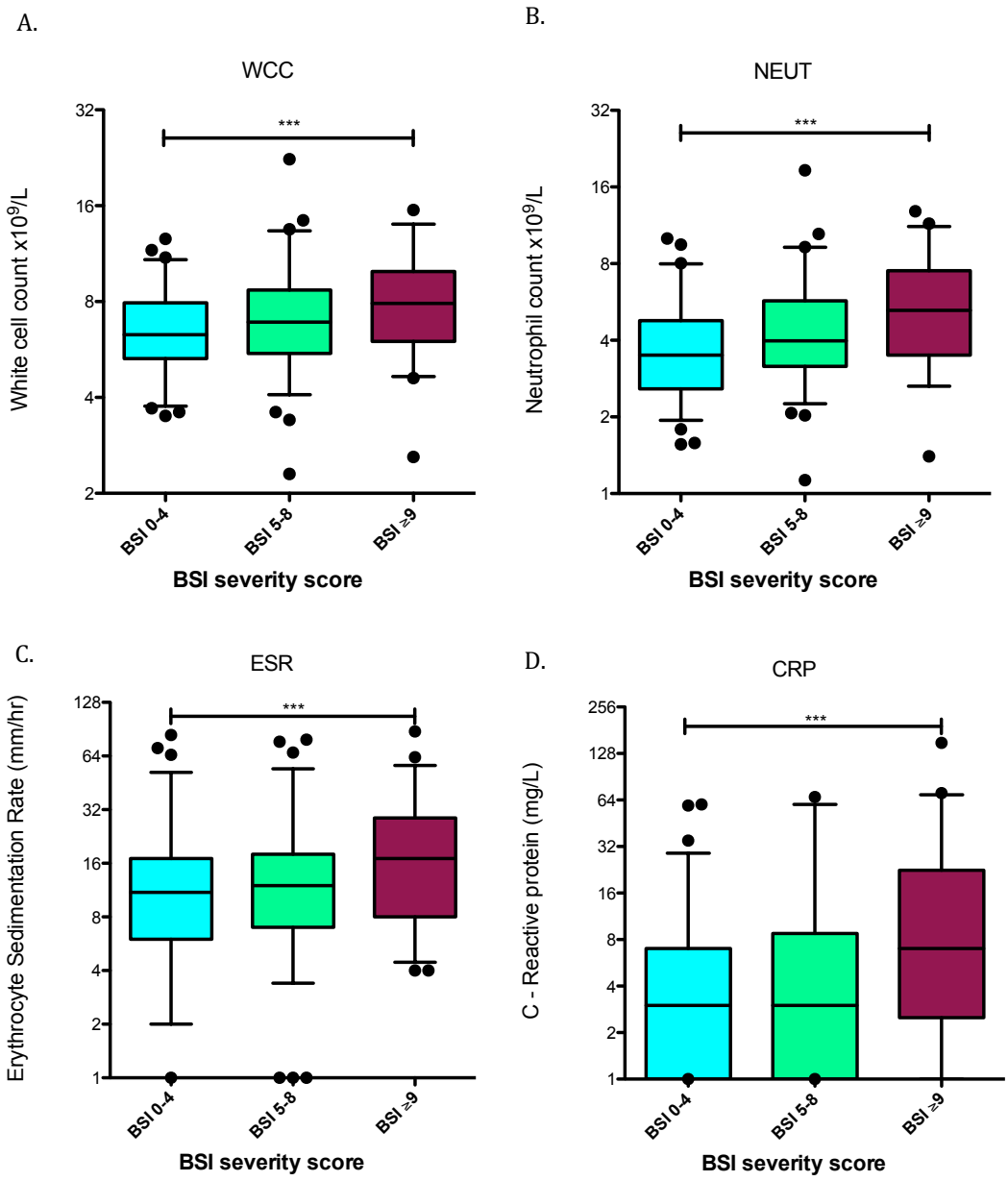


Figure 1. Blood inflammatory markers in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers of 5-95% CI and dots of outliers. A: White cell count p= 0.0084, B: Neutrophil count p=0.0015, C: Erythrocyte sedimentation rate p=0.0065, D: C-reactive protein p=0.0045, ***p<0.001.

3.3.3 Sputum inflammatory markers results

Free neutrophil elastase (NE), myeloperoxidase (MPO) and interleukin-8 (IL-8) was investigated in groups as per increasing BSI severity score. NE median (IQR) results for groups 0-4, 5-8 and ≥9 were 2569 UG/ml (1408-6191), 2600 UG/ml (1915-3404) and 3238 UG/ml (2013-11552) respectively, p=0.076. MPO median (IQR) results for groups 0-4, 5-8 and ≥9 were 1719 UG/ml (859-4718), 1125 UG/ml (399-3096) and 2521 UG/ml (1159-9544) respectively, p=0.002. IL-8 median (IQR) results were 14976 pg/ml (3815-26466), 13257 pg/ml (6324-30975) and 11848 pg/ml (5935-31455) respectively, p=0.6. There was no association with NE and IL-8. There was statistical significance with MPO –figure 2.

Figure 2.

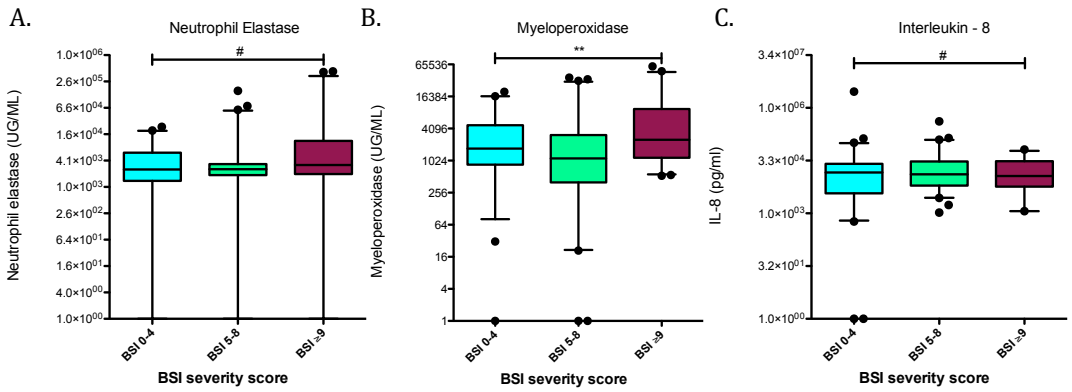


Figure 2. Sputum inflammatory markers in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers 5-95% CI and dots of outliers, A: Neutrophil elastase #p=0.076, B: Myeloperoxidase **p=0.01, C: Interleukin - 8 #p= 0.57.

3.3.4 Sputum characteristics

There were associations with increasing 24hr sputum volume and with increasing sputum purulence but not with spontaneous sputum volume (sputum collected within 4hrs of waking) with higher BSI scores – see Figure 3.

Figure 3.

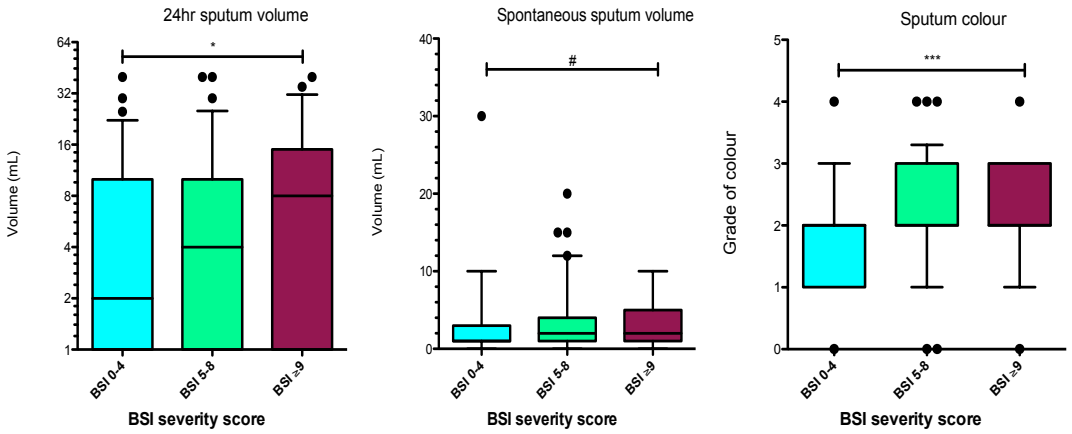


Figure 3. Sputum properties in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers 5-95% CI and dots of outliers. A: 24hr sputum volume p=0.02, B: Spontaneous sputum p=0.76, C: Sputum colour p= 0.0008, *p<0.05, ***p<0.001, #p>0.05.

3.3.5 Incremental shuttle walk test

The distance walked in the incremental shuttle walk test was significantly lower in the progressive BSI tertiles of severity. In BSI group 0-4 the median (IQR) ISWT distance was 470m (365-580m), for group 5-8 was 350m (260-485m) and for those with a score of 9 and over had deteriorated to 250m (95-365m), p<0.0001 – figure 4.

Figure 4.

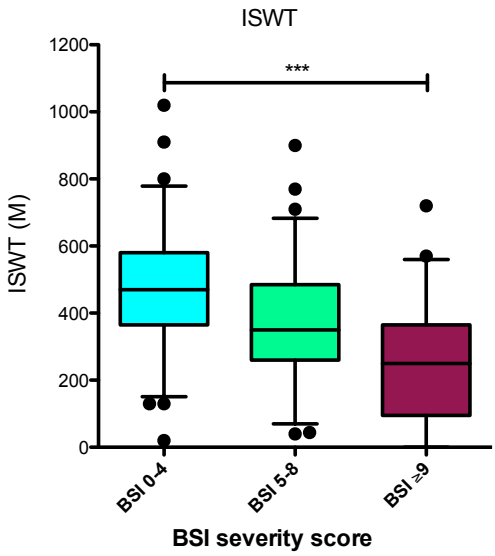


Figure 4. Incremental shuttle walk test in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers 5-95% CI and dots of outliers, ***p<0.0001.

3.3.6 Bacteriology

Quantitative bacteriology was performed on all sputum samples. There was an increase in bacterial load with increasing severity of BSI from groups 0-4, 5-8 and ≥9 with median (IQR) counts of 0 CFU/ml (0 - $9.3x10^6$), $0.1x10^5$ CFU/ml (0 – $7.4 x10^6$) and $1.27x10^7$ CFU/ml ($0.02 x10^6$ – $1.0 x10^8$) respectively, p<0.0001 –figure 5.

Figure 5.

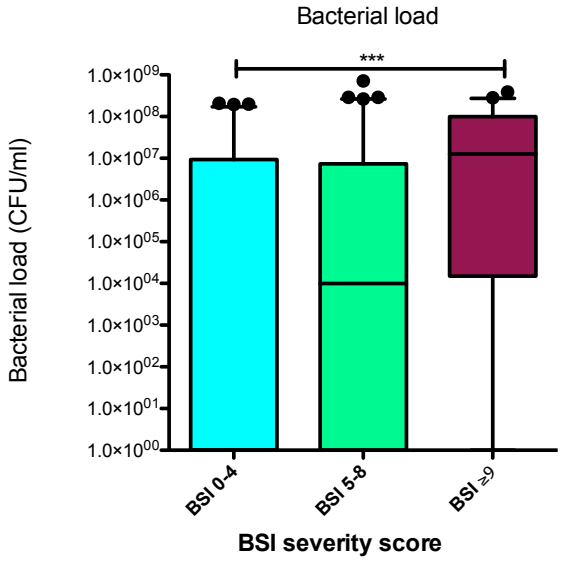


Figure 5. Bacterial load in stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers 5-95% CI, dots of outliers, ***p<0.0001.

3.3.7 Lung function tests results

Forced expiratory volume in 1 second (FEV_1), percent predicted of FEV_1 (% FEV_1), forced vital capacity (FVC), percent predicted FVC (%FVC), forced expiratory flow at 25-75% (FEF_{25-75}) and percent predicted of FEF_{25-75} (%FEF_{25-75}) was tested in all patients. There was an association with all markers of lung function and increasing severity across the BSI groups, $p<0.0001$ – see figure 6. Median (IQR) for each BSI group 0-4, 5-8 and ≥9 for FEV_1: 2.32L (1.8 - 2.9), 1.86L (1.4 - 2.4) and 1.48L (1.1 – 2.1); %FEV_1: 90% (75 - 100), 81% (64 - 98) and 60.5% (48 – 78); FVC: 3.4L (2.7 – 4.1), 3.0L (2.4 – 3.6) and 2.6L (2.0 – 3.1); % FVC: 101% (88 - 117), 99% (84 - 114) and 79% (65 - 97); FEF_{25-75}: 1.5L (0.9 – 2.7), 1.0L (0.6 – 1.7) and 0.67L (0.44 – 1.2); %FEF_{25-75}: 46% (28.8 – 73.3), 36% (22 - 55) and 25% (16 - 40) respectively.

Figure 6.

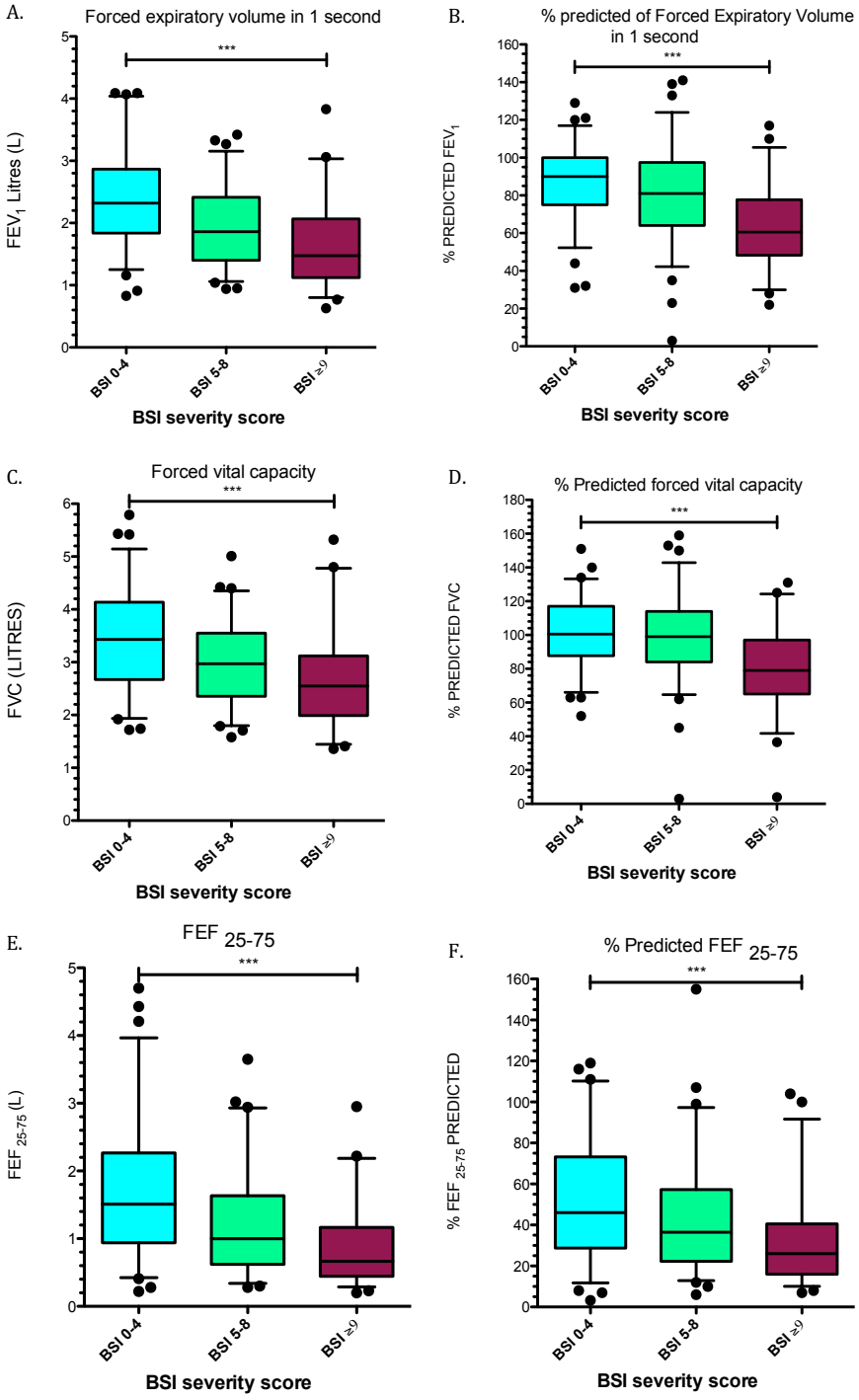


Figure 6. Lung function tests in clinically stable patients with 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR), whiskers 5-95% CI, dots of outliers. A: FEV_1 (L) B: % predicted FEV_1, C: FVC (L), D: % predicted FVC, E: FEF_{25-75}, F: % predicted FEF_{25-75}, ***P<0.0001.

3.3.8 Quality of life results

The Leicester Cough Questionnaire (LCQ) assesses quality of life based on the impact of cough severity across the domains of physical, psychological and social items. The St George's Respiratory Questionnaire (SGRQ) assesses quality of life based on symptoms, activity and psychosocial impact. The median (IQR) of LCQ results for each BSI tertile were: 19 (16-20), 18 (14-20) and 16 (13-19) respectively. The median (IQR) for the SGRQ for each group was: 26 (17-37), 28.5 (16-44) and 44 (31-66) respectively. Both quality of life assessments correlated with BSI severity; SGRQ: p<0.0001 and the LCQ: p=0.01 – see figure 7.

Figure 7.

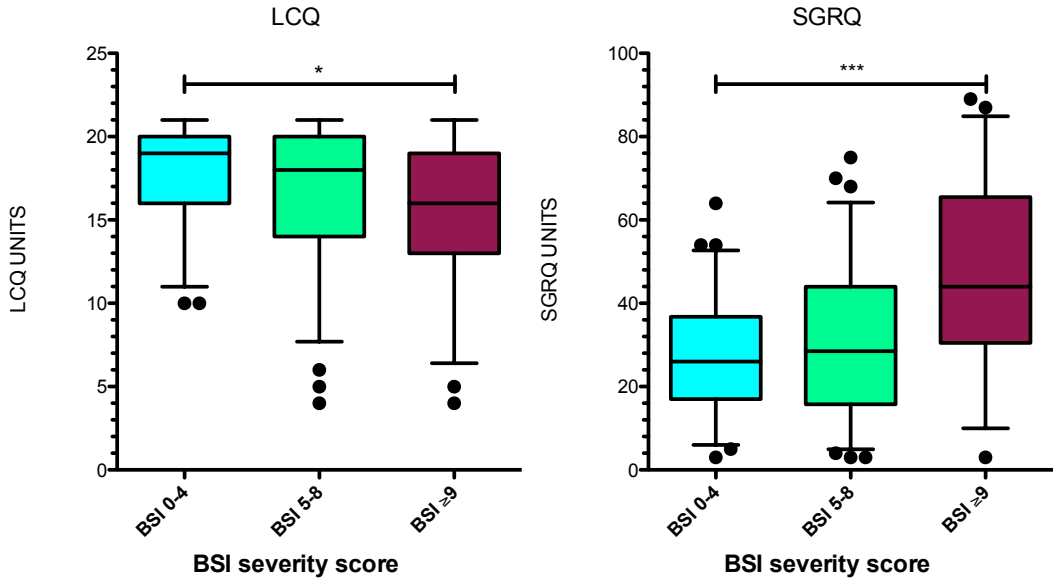


Figure 7. Quality of life in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' Bronchiectasis Severity Index groups shown as boxplot with median (IQR) and 5-95% CI, A: Leicester Cough Questionnaire (LCQ), *p=0.01, B: St George's Respiratory Questionnaire (SGRQ), ***p<0.0001.

3.4 Discussion

The bronchiectasis severity index score is internationally validated for predict 30-day mortality rate and hospitalisation. Work from this thesis supports that in addition the BSI links with sputum purulence, 24hr sputum volume, quantitative bacterial load, lung function, functional capacity, quality of life, sputum and serum inflammatory markers. These other endpoints have been shown to be of importance previously but have not to date been linked with the Bronchiectasis Severity Index.

Sputum colour was investigated by Murray *et al* in stable patients with bronchiectasis. They reported good reliability of sputum colour between patients and clinician and that it was predictive of bacterial colonisation ($p<0.0001$). They also reported independent factors associated with sputum purulence were bacterial colonisation, radiological varicose or cystic bronchiectasis, FEV_1 less than 80% predicted and diagnosis of bronchiectasis aged less than 45yrs old (Murray *et al*, 2009).

Bacterial load has been evaluated with conflicting evidence in the literature. Tunney and colleagues reported that bacterial load did not change with exacerbations although they did not measure bacterial load during clinical stability and during an exacerbation in the same patients and their study on exacerbations was small (n=14) (Tunney *et al*, 2013). Chalmers and colleagues reported there was a direct relationship between bacterial load and the severity of exacerbations and risk of subsequent exacerbations. They linked bacterial load with airways inflammation and systemic inflammation when assessing both inpatients and outpatient exacerbations. Furthermore, they reported the reduction of bacterial load with short and long term antibiotic therapy (Chalmers *et al*, 2012).

Lung function in bronchiectasis has not been very well characterised in bronchiectasis. A study by Guan and colleagues have reported in 142 stable patients that the variables associated with a 50% or lower FEV_1 were radiological severity ($p<0.01$), presence of *Pseudomonas aeruginosa* ($p<0.01$) and the presence of symptoms for 10 or more years ($p=0.01$). They also noted differences in FEV_1 and

FVC between exacerbations and convalescence in a small subgroup ($p<0.05$). Martinez-Garcia and colleagues also investigated FEV_1 decline in 76 stable patients. They also found accelerated decline in function to be associated with more severe exacerbations, the presence of *Pseudomonas aeruginosa* in sputum and systemic inflammation (Martinez-Garcia *et al*, 2007).

The ISWT is an objective measurement of functional capacity and has been previously shown to remain stable in patients with clinical stability i.e. no change to medications and no exacerbation 6 months apart (Mandal *et al*, 2014). It has also been shown to improve with long-term nebulised antibiotics (Murray *et al*, 2011) and anti-inflammatory treatment (Mandal *et al*, 2014). Work from this thesis demonstrated that the ISWT mean distances deteriorated significantly with increasing severity of BSI. Work from this thesis has validated the ISWT as a clinical endpoint in bronchiectasis and found a 5% difference in distance walked to be the minimum clinically important difference (MCID) (See chapter 5) (Cartlidge *et al*, 2018).

The Leicester Cough Questionnaire (LCQ) and the St George's Respiratory Questionnaire (SGRQ) have both been validated as clinical endpoints for use in bronchiectasis (Murray *et al*, 2009d Wilson *et al*, 1997b). The MCID for the LCQ is 1.3units and for the SGRQ is 4units.

Previous studies have shown the importance of airway inflammation and its correlation with higher bacterial loads and subsequent increased risk of exacerbation and with severe exacerbations (Chalmers *et al*, 2012). Airway inflammatory markers have also been shown to improve with the use of long-term antibiotic use (Murray *et al*, 2011 and Chalmers *et al*, 2012) They also reported on the increased airways inflammation present with patients colonised with *Pseudomonas aeruginosa* and in those with more severe radiological bronchiectasis (Chalmers *et al*, 2012). Elevation of Neutrophil elastase, a product of activated neutrophils has been investigated by Chalmers and colleagues and was found to be associated with the bronchiectasis severity index score ($r=0.49$, $p<0.0001$) but was not independently associated with

mortality rates. The authors found neutrophil elastase to rise in exacerbations and have good discrimination for severe exacerbations and all-cause mortality (Chalmers *et al*, 2016).

Relatively little is known about systemic inflammation in patients with bronchiectasis due to the paucity of studies in this area. A study by Wilson *et al* in 87 stable bronchiectasis patients investigated the correlation of inflammatory markers with radiological severity, lung function, sputum bacteriology and health related quality of life as assessed by the SGRQ. They reported patients have raised inflammatory markers when clinically stable and that inflammatory markers correlate with disease severity; WCC, neutrophil count, ESR and CRP all correlated with radiological severity, WCC and CRP correlated with total SGRQ scores (Wilson *et al*, 1998).

3.5 Significance

There have been no prior studies linking the BSI with sputum purulence despite its importance in recognising severity. This is the first study to show increasing sputum purulence, a very quick and easy assessment, links with increasing severity of BSI. The role of quantitative bacteriology has been debated in the literature but this study supports the notion that increasing bacterial load is associated with increased severity of bronchiectasis. The most commonly research lung function parameter is FEV_1 and variables associated with its decline have been investigated. The BSI already incorporates percent predicted FEV_1 as part of its severity scoring system but as this can be affected by other respiratory conditions that patients with bronchiectasis might have such as asthma and COPD, the other measures of lung function were explored. This is the first study to find all parameters of actual and predicted FEV_1, FVC and FEF 25-75 correlated with worsening severity of bronchiectasis as assessed by the BSI. Functional capacity in bronchiectasis has not been well studied in bronchiectasis and has only recently been validated in this respiratory condition (Cartlidge *et al*, 2018). This study has shown that the ISWT links with BSI severity in stable patients. The LCQ and SGRQ are validated clinical endpoints in bronchiectasis. This is the first study to show the LCQ measurement of

quality of life links with BSI severity scoring. Lastly, the BSI is a very clinical tool and does not incorporate any laboratory endpoints that assess inflammation. This study demonstrates that markers of serum inflammation –WCC, Neutrophil count, ESR and CRP all increase with worsening severity of bronchiectasis as assessed by the BSI. The changes in the serum inflammatory markers, whilst statistically significant are small and therefore of questionable clinical significance.

3.6 Limitations

The BSI score has already been externally validated and predicts mortality and hospitalization. This study reiterates the current literature findings that this scoring system is robust in this outpatient cohort. Several clinical markers are already included in the BSI but this study shows additional markers like exercise tolerance (ISWT) and sputum colour correlate well with it. Due to the small number of patients in this study, the added value of these endpoints to BSI to predict 30-day mortality and hospitalisation could not be explored.

3.7 Conclusion

The Bronchiectasis Severity Index stratifies patients into tertiles and has been shown to predict 30day mortality and hospitalization. The authors have shown the BSI scoring of severity to also link with other endpoints of increasing sputum purulence, 24hr sputum volume, bacterial load, functional capacity as assessed by the ISWT, quality of life, lung function parameters of FVC and FEF_{25-75} in addition to FEV_1, and serum inflammatory markers. Further work is needed to assess whether these clinical endpoints could be additive or independent to the BSI in predicting 30-day mortality and future hospitalisation.

Chapter 4:
Defining the characteristics of outpatient exacerbations

4.1 Introduction to exacerbations

Bronchiectasis is a chronic respiratory condition where patients suffer from a daily cough, sputum production and breathlessness. It is a heterogeneous condition with multiple aetiologies and the severity of the condition can range from mild to severe. Patients can suffer from recurrent exacerbations which necessitates treatment; mainly in the form of short term antibiotics and chest physiotherapy. Patients can however also suffer from daily symptoms when clinically stable that do not necessitate acute treatment but for which long term treatments such as anti-inflammatories, prophylactic antibiotics, mucolytic therapy could be explored. As with any chronic progressive lung disease the distinction between the patient's baseline, albeit far off a healthy person's baseline with an exacerbation can be blurred and hence the decision to treat can be difficult.

The British Thoracic Guidelines defined an exacerbation as an acute deterioration (usually over several days) with worsening local symptoms (cough, increase sputum volume or change of viscosity, increased sputum purulence with or without increasing wheeze, breathlessness, haemoptysis) and/or systemic upset (Pasteur *et al*, 2010). There has recently been a European consensus led by Hill and colleagues to define an exacerbation for use in clinical research trials. They concluded an exacerbation to be when a patient exhibits a deterioration in three of more of the following key symptoms for at least 48hours: cough; sputum volume and/or consistency; sputum purulence; breathlessness and/or exercise tolerance; fatigue and/or malaise; haemoptysis AND a clinician determines that a change in bronchiectasis treatment is required (Hill *et al*, 2017).

Exacerbations meeting these criteria treated as outpatients are less severe and treated with oral antibiotic therapy. The British Thoracic Society guidelines recommends that intravenous antibiotic therapy should be considered when patients are particularly unwell, have resistant organisms or have failed to respond to oral therapy (Pasteur *et al*, 2010).

There are a paucity of studies investigating outpatient exacerbations of bronchiectasis and the mechanism underpinning this needs further study.

The role of quantitative bacteriology and bacterial load in exacerbations of bronchiectasis is unclear with previous literature offering different views. Chalmers and colleagues suggest bacterial load in the stable state directly correlates with markers of airway inflammation and the risk of subsequent exacerbations and severe exacerbations. They also suggested that intravenous antibiotics reduced bacterial load in addition to airway and systemic inflammation. Tunney *et al* investigated bacterial load using quantitative microbiology as well as 16S rDNA pyrosequencing and found similar communities of microbial taxa (113 distinct microbial taxa) were present in low abundance when clinically stable, start of exacerbation and after treatment. The clinical significance of these numerous taxa in low abundance is unclear. To further investigate the role of bacterial load we aimed to perform qualitative and quantitative microbiology on patients sequentially at stable baseline, start and end of exacerbation in the same patients. The hypothesis is that an exacerbation with a rise in bacterial load of 1 or more log unit in colony forming units per ml would lead to a more severe exacerbation.

This chapter aims to provide further guidance on the phenotype of outpatient exacerbations and to investigate the significance of bacterial load rise in exacerbations of bronchiectasis.

4.2 Clinically unstable patients

94 of the 207 patients that completed a baseline visit went on to have an ‘exacerbation’ during the length of the study. The study did specify that patients should be seen for their first exacerbation after their baseline visit in the study and that subsequent exacerbations would not be included. Due to the length of the study (2 years) and the limitations (patients could only be seen during working hours) there will undoubtedly have been patients that sought medical advice from their general practitioner, emergency services or started rescue medications at home.

Patients completed all tests at a 'baseline' visit (Visit 0, V0) when clinically well and free from any exacerbating symptoms or antibiotic use in the last 6 weeks. The results were recorded and patients were then encouraged to get in touch when they felt they had symptoms of an exacerbation. A suitable time would be arranged (often the same day but occasionally the next day) and the patient would return for a 'Start of exacerbation' visit (Visit start, VS) and repeat all tests. The patient would be issued with a 14-day prescription of antibiotics, according to sensitivities of any previous microbiological sputum results, as per unit guidelines. After 14 days patients would return for an 'End of exacerbation' visit (Visit end, VE) and repeat all tests and to ensure clinical recovery.

4.3 Methodology

4.3.1 Ethics

Ethical approval was granted by the West of Scotland Research ethics service, REC reference 13/WS/0230. All patients provided informed written consent and were recruited from a single centre.

4.3.2 Study design

Patients were recruited from a routine bronchiectasis clinic in a single tertiary specialist centre. Patients over the age of 16 with clinically evident bronchiectasis, previously diagnosed by high resolution computer tomography (CT) scan. Patients were asked to attend for three visits –baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Patient were asked to attend an outpatient appointment when clinically stable (no antibiotic use in the last 6 weeks) where baseline assessments (see below) were performed. They were then given a dedicated phone number for the clinical researcher and advised to phone when they felt they were experiencing an exacerbation. After discussion, if the clinical fellow thought antibiotics were warranted then the patients were seen for the second visit. All assessments were repeated and a prescription for 14 days of antibiotics as per their previous microbiology results was given. After 14 days patients re-attended for the final visit and assessments were repeated.

4.3.3 Incremental shuttle walk test

The test was performed according to standard test procedure (Singh *et al*, 1992) and consisted of a 10-metre shuttle course on a flat surface with the walking speed controlled using pre-recorded audio signals. The test continues until the participant is unable to complete the shuttle in the designated time or the patient decides to stop due to symptoms (e.g. breathless, leg fatigue). The distance completed within the timeframe was recorded. The successful completion of distance walked in our study was recorded in increments of 10m.

4.3.4 Lung function

We measured pre-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and ratio FEV_1:FVC by spirometry according to standardized guidelines [ATS guidelines]. Patients were asked to take three attempts and the best results were recorded. All patients have previously performed spirometry as part of routine clinical practise.

4.3.5 Sputum

Sputum colour was graded according to a visual colour chart rating. Mucoid sputum was rated 1, mucopurulent rated as 2 and purulent sputum rated as 3 or 4 depending on colour (Murray *et al*, 2009). 24hour sputum volume was recorded by asking patients to expectorate solely into a universal container for 24hours. Qualitative and quantitative microbiology was performed on all samples at baseline, pre- and post 14 days of antibiotics.

4.3.6 Qualitative and quantitative microbiology (Murray *et al*, 2011)

For analysis culture results were grouped as either positive for '*Pseudomonas aeruginosa'*, 'potentially pathogenic microorganisms' (*Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus*) or 'other gram negatives' (*Enterobacter, Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia, Serratia marcescens, Proteus mirabilis, Acinetobacter baumannii, Achromobacter xylosoxidans, Citrobacter freundii and Aeromonas hydrophila*) and 'mixed normal flora' (no dominant pathogen cultured

or presence of respiratory commensal flora only).

4.3.7 Sputum inflammatory markers

Whole sputum samples were also centrifuged to produce supernatants on which myeloperoxidase (MPO), neutrophil elastase (NE) and interleukin – 8 (IL-8) were tested. MPO (Steinman *et al*, 1972) was assayed using a chromogenic substrate assay (Stockley *et al*, 2000), free neutrophil elastase (NE) (Buttle *et al*, 1990) was measured by spectrophotometry with a synthetic substrate (methoxysuccinyl-Ala-Ala-Pro-Val paranitroanilide; Sigma, Gillingham, UK) were tested as per standard protocol (Hill *et al*, 1999, Hill *et al*, 2000) and interleukin – 8 (IL-8) was assayed using commercially available specific ELISAs (R&D Systems, Oxford, UK).

4.3.8 Blood inflammatory markers

Venous blood sampling was taken to monitor full blood count, urea and electrolytes, Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

4.3.9 Statistical analysis

All data were analysed using Graphpad prism version 5.0a (Graphpad software, San Diego, CA, USA). For demographic and clinical variables, data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables unless specified otherwise. A P value <0.05 was considered statistically significant for each analysis. Comparison of changes within a group i.e. before and after treatment was calculated using Wilcoxon signed rank test and we compared categorical data between groups was calculated using the $\chi 2$ test.

4.4 Results

4.4.1 Baseline characteristics

The 94 patients with exacerbations within the study that completed a start of exacerbation and end of exacerbation visit, had the following characteristics (Table 1) and aetiology (Table 2):

Table 1.

Characteristic	Exacerbations	No exacerbations
Number	94	113
Age (yrs) (mean, (SD))	65.76 (9.9)	65.82 (11.2)
Female	51(54)	59 (52)
Current smokers	2 (2)	7 (6)
Ex-smokers	35 (37)	48 (42)
Never	57 (61)	58 (51)
BMI Kg/m^2 (mean (SD))	32.39 (53.79)	26.33 (4.96)
COPD	13 (14)	22 (19)
Asthma	16 (17)	7 (6)
Interstitial lung disease	0	3 (3)
Lung cancer	2 (2)	0
Pulm. Hypertension	0	1 (1)
Inactive ABPA	11 (12)	10 (9)
IHD	5 (5)	9 (8)
Other cancers	7 (7)	15 (13)
LTOT	0	2 (2)
Inhaled steroid	16 (17)	7 (6)
Dose (mean, (SD))	216mcg (118mcg)	200mcg (70.7mcg)
Oral steroid	2 (2)	10 (9)
Dose (mean, (SD))	10mg (7mg)	7.2mg (5.1mg)
Nebulised bronchodilators	2 (2)	5 (4)
Long-term oral antibx	2 (2)	2 (2)
Long-term inh antibx	5 (5)	2 (2)
Cyclical IV antibiotics	1 (1)	0
Bronchiectasis severity	6.9 (4.4)	6.5 (4.1)
FEV_1	2.02 (0.75)	2.05 (0.79)
% pred FEV_1	79.2 (24.9)	77.5 (24.6)

Baseline characteristics of patients that did and did not present with an exacerbation. N (%) unless otherwise stated. Body mass index (BMI), Chronic obstructive pulmonary disease (COPD), Allergic bronchopulmonary aspergillosis (ABPA), Ischaemic heart disease (IHD), Long term oxygen therapy (LTOT), inhaled antibiotics (inh antibx) and forced expiratory volume in 1 second (FEV_1).

Table 2.

Aetiology	Exacerbation N (%)	No exacerbation N (%)
Idiopathic	37 (39)	56 (50)
Post infectious	29 (31)	26 (23)
COPD	1 (1)	8 (7)
Inflammatory bowel disease	2 (2)	5 (4)
Immunoglobulin deficiency	4 (4)	5 (4)
Allergic bronchopulmonary aspergillosis	6 (6)	6 (5)
Connective tissue disease	4 (4)	3 (3)
Aspiration or inhalation or GORD	11 (12)	4 (4)

Table to show the N (%) of patients with different aetiologies of bronchiectasis. COPD: chronic obstructive pulmonary disease, GORD: gastric oesophageal reflux disease.

4.4.2 Lung function

4.4.2.1 Forced expiratory volume in 1 second (FEV_1)

94 patients performed spirometry at baseline and start of exacerbation. 93 patients performed spirometry at the end of an exacerbation. Results are outlined in table 3.

Table 3.

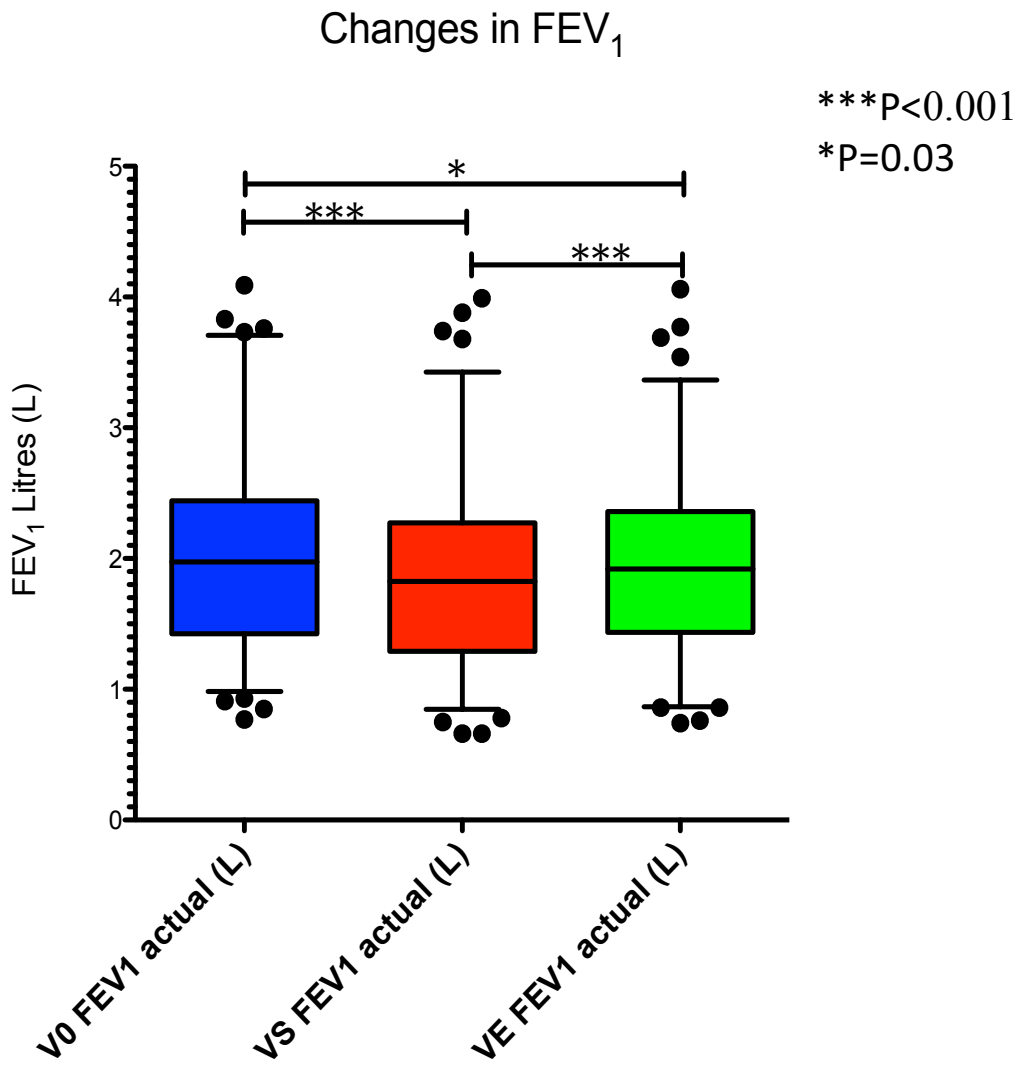
	Baseline (VO) FEV_1 Litres (L)	Start of exacerbation (VS) FEV_1 Litres (L)	End of exacerbation (VE) FEV_1 Litres (L)
No. of values	94	94	93
25% percentile	1.425	1.290	1.435
Median	1.975	1.825	1.920
75% percentile	2.443	2.273	2.360

Table to show the change in forced expiratory volume in 1 second (FEV_1) in patients when stable (VO) and with an exacerbation (VS and VE).

The median value for FEV_1 at baseline as 1.98L which reduced by 150ml to 1.83L at start of exacerbation. Following a 14-day course of antibiotics the median FEV_1 increased by 95ml to 1.92L. Patient's FEV_1 median value at the end of exacerbation was 55mls short of the median baseline value.

A Wilcoxon paired t test analysis was performed. The reduction in FEV_1 from baseline visit (VO) to start of exacerbation (VS) was statistically significant ($P<0.0001$). The increase in FEV_1 from start of exacerbation to end of exacerbation was also statistically significant ($P<0.001$). The median FEV_1 at end of exacerbation was also found to be significantly lower than initial baseline tests ($P=0.03$). See figure 1 for schematic.

Figure 1.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in forced expiratory volume in 1 second (FEV_1) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, *p<0.05, ***p<0.001.

4.4.2.2 Percentage of predicted forced expiratory volume in 1 second (% FEV_1)

94 patients performed spirometry at baseline and start of exacerbation. 93 patients performed spirometry at the end of an exacerbation. Results for percent predicted of FEV_1 are outlined in table 4.

The median value for percentage of predicted FEV_1 was 81% at baseline. This reduced to a median value of 78% at the start of exacerbation. After a course of antibiotics, the median value for percent predicted remained the same at 78%. There was a 3% reduction in the median percent predicted of FEV_1 at the end of exacerbation when compared with baseline values.

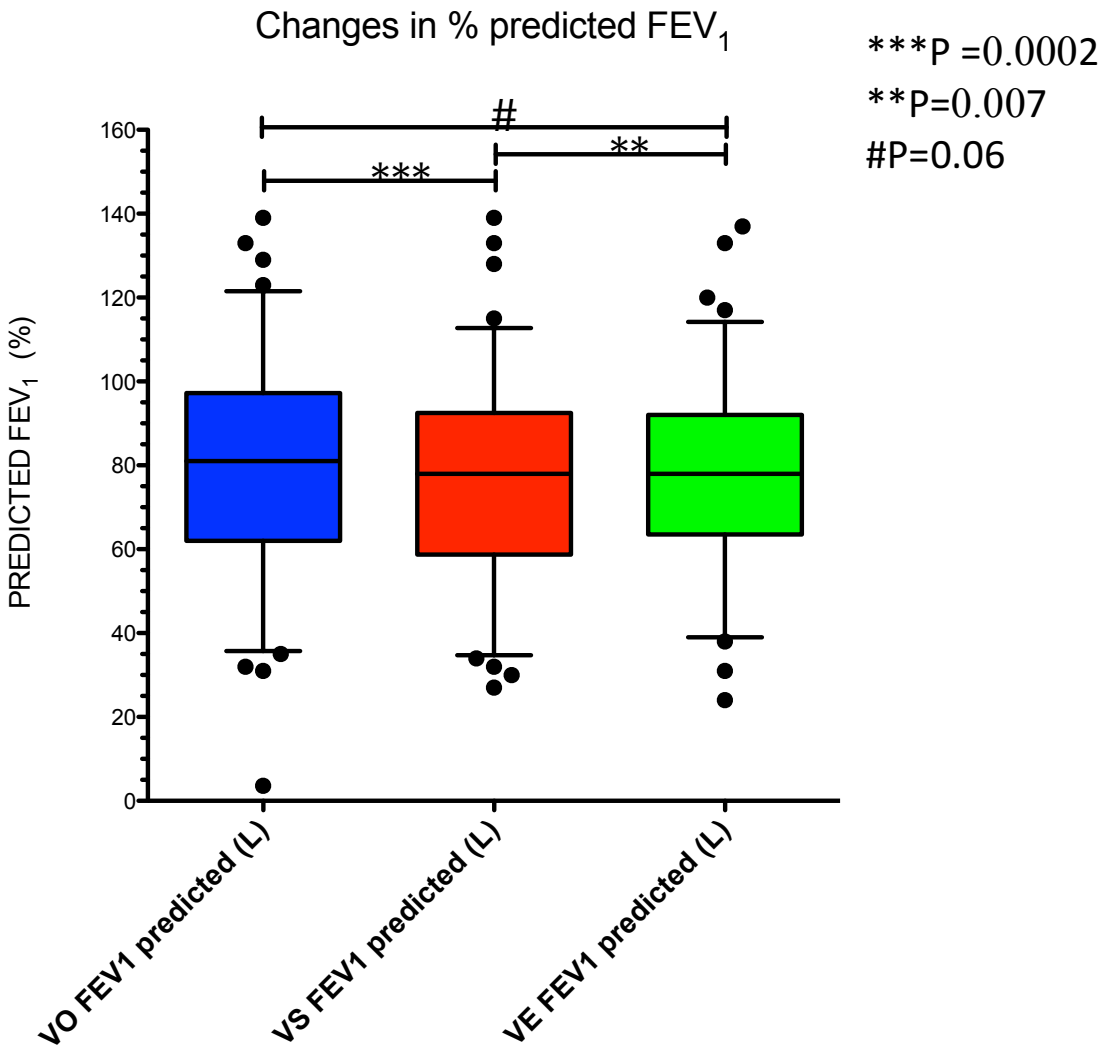
Table 4.

	Baseline (VO) % predicted FEV_1 (%)	**Start of exacerbation (VS) % predicted FEV_1 (%)**	**End of exacerbation (VE) % predicted FEV_1 (%)**
No. of values	94	94	93
25% percentile	62.00	58.75	63.50
Median	81.00	78.00	78.00
75% percentile	97.25	92.50	92.00

Table to show the change in percent predicted of FEV_1 in patients when stable (VO) and with an exacerbation (VS and VE).

The reduction in percent predicted FEV_1 from baseline to start of exacerbation was statistically significant when analysed using a paired Wilcoxon statistical analysis (P=0.0002). There was also a statistically significant increase in percent predicted FEV_1 from start of exacerbation to end of exacerbation with the mean increasing from 75.9% to 77.8%, although the median values remained the same. There was no significant difference from baseline values to end of exacerbation and may suggest that patients returned to baseline level (Figure 2).

Figure 2.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in percent predicted in FEV_1 (%) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, **p<0.01, ***p<0.001, #p>0.05.

4.4.2.3 Forced vital capacity (FVC)

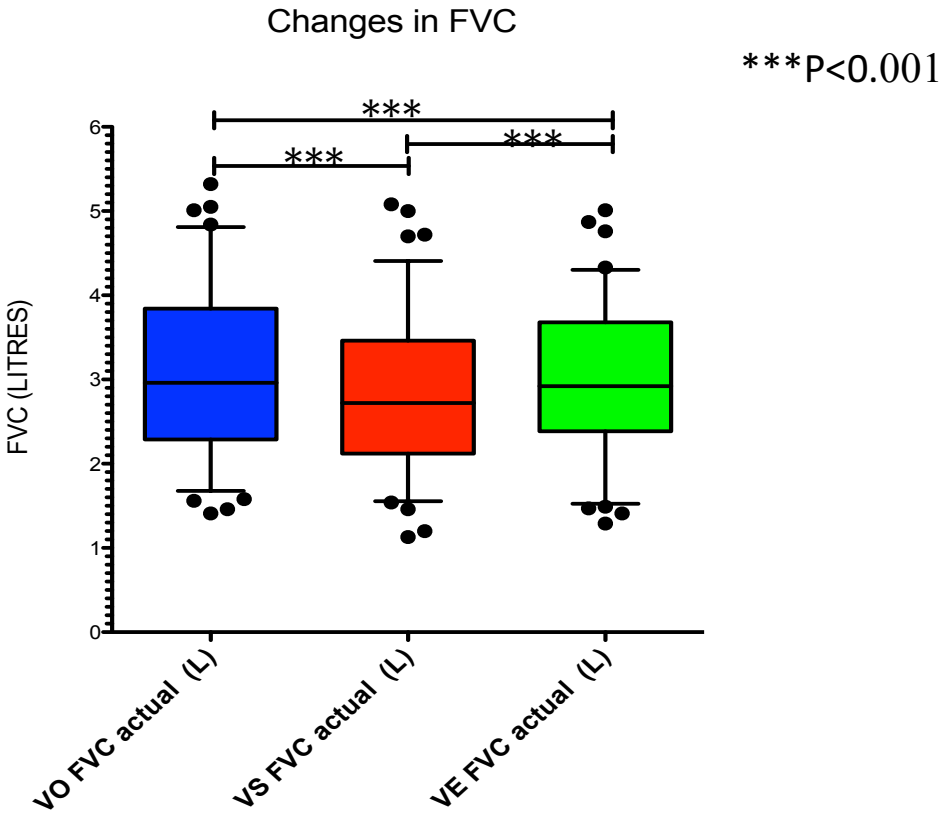
94 patients performed spirometry at baseline and start of exacerbation. 93 patients performed spirometry at the end of an exacerbation. Results are outlined in table 5. The median forced vital capacity at baseline level is 2.96L. This decreases by 240ml at start of exacerbation. After 14 days of antibiotics the median FVC increases by 200mls to 2.92L. The end of exacerbation median value is 40mls less that the initial baseline level.

Table 5.

	Baseline (VO) FVC (L)	Start of exacerbation (VS) FVC (L)	End of exacerbation (VE) FVC (L)
No. of values	94	94	93
25% percentile	2.288	2.120	2.385
Median	2.960	2.720	2.920
75% percentile	3.843	3.463	3.680

Table to show the change in forced vital capacity (FVC) in patients when stable (VO) and with an exacerbation (VS and VE).

Figure 3.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in forced vital capacity (FVC) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, ***p<0.001.

Statistical analysis shows a significant reduction in FVC from baseline to start of exacerbation. 14 days of antibiotics results in a significant improvement but not enough to reach initial baseline level. There remains a significant difference between baseline and end of exacerbation forced vital capacity (Figure 3).

4.4.2.4 Percentage predicted forced vital capacity (% FVC)

94 patients performed spirometry at baseline and start of exacerbation. 93 patients performed spirometry at the end of an exacerbation. Results for percent predicted of forced vital capacity are outlined in table 6.

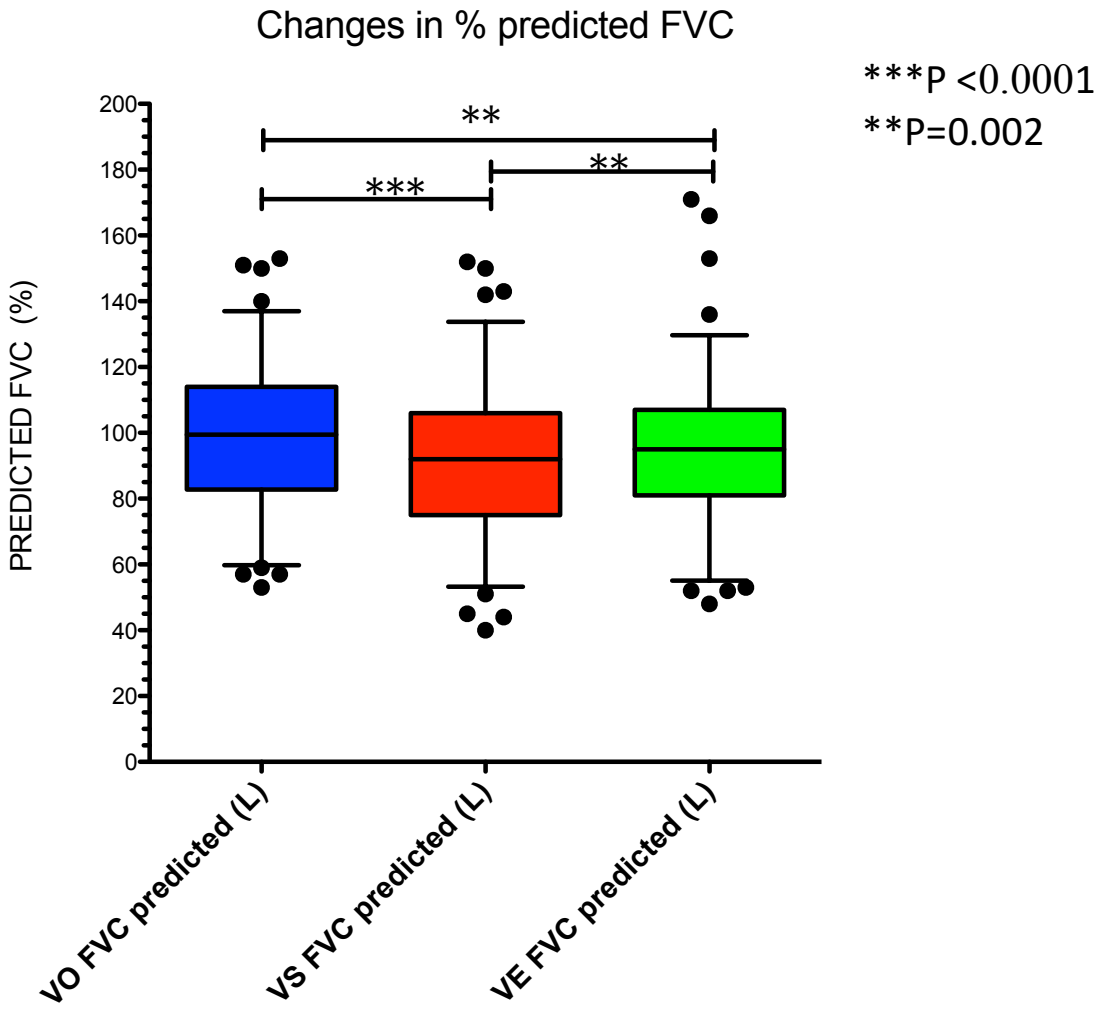
The median value for percent predicted of forced vital capacity at baseline was 99.5%. This reduced by 7.5% to 92% when patients self-presented with an exacerbation of bronchiectasis. Following therapy, the percent predicted increased by 3%. There remained a 4.5% reduction in percent predicted FVC at end of exacerbation when compared with baseline.

Table 6.

	Baseline (VO) % predicted FVC (%)	**Start of exacerbation (VS) % predicted FVC (%)**	**End of exacerbation (VE) % predicted FVC (%)**
No. of values	94	94	93
25% Percentile	82.75	75.00	81.00
Median	99.50	92.00	95.00
75% Percentile	114.0	106.0	107.0

Table to show the change in percent predicted of forced vital capacity (FVC) when stable (VO) and with an exacerbation (VS and VE).

Figure 4.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in percent predicted forced vital capacity (FVC) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance,p<0.01, ***p<0.001.**

There is a significant reduction in the percent predicted forced vital capacity at start of exacerbation when compared with baseline (P<0.0001). The percent predicted significantly improves at end of exacerbation with treatment (P=0.002) but does not reach initial levels with a significant reduction remaining when compared with baseline results (P=0.002). See figure 4.

4.4.2.5 Forced expiratory flow$_{25-75}$ (FEF_{25-75})

93 patients performed spirometry at baseline and start of exacerbation. 91 patients had FEF_{25-75} measured at start and 93 at end of exacerbation. The reduced number of results was due to equipment failure. Results for forced expiratory flow$_{25-75}$ and percent predicted FEF_{25-75} are outlined in table 7 and table 8.

Forced expiratory flow$_{25-75}$ measures the volume expired in mid expiration when maximally breathing out. Results showed a small reduction of 50mls in volume expired at start of exacerbation when compared with baseline. This reduction disappeared at the end of exacerbation with median values of 1.07L expired at both baseline and end of exacerbation. The results did not reach statistical significance.

Similarly, the percent predicted FEF_{25-75} reduced from 37% at baseline to 36% at start of exacerbation. After treatment with 14 days of antibiotics, the percent predicted median value was 35%. The results did not reach statistical significance.

Table 7.

	Baseline (VO) FEF_{25-75} (L)	Start of exacerbation (VS) FEF_{25-75} (L)	End of exacerbation (VE) FEF_{25-75} (L)
No. of values	93	91	93
25% percentile	0.6050	0.5900	0.6250
Median	1.070	1.020	1.070
75% percentile	1.695	1.510	1.600
P value compared with VO		0.9	0.2
P value compared with VS			0.07

Table to show the change in forced expiratory flow$_{25-75}$ (FEF_{25-75}) when stable (VO) and with an exacerbation (VS and VE).

Table 8.

	Baseline (VO) % predicted FEF_{25-75}	Start of exacerbation (VS) % predicted FEF_{25-75}	End of exacerbation (VE) % predicted FEF_{25-75}
No. of values	93	91	93
25% percentile	21.00	22.00	24.50
Median	37.00	36.00	35.00
75% percentile	58.50	52.00	56.00
P value compared with VO		0.9	0.3
P value compared with VS			0.1

Table to show the change in percent predicted forced expiratory flow$_{25-75}$ (FEF_{25-75}) when stable (VO) and with an exacerbation (VS and VE).

4.4.3 Sputum characteristics

All patients were asked to provide a spontaneous sputum sample within 4 hours of waking in the morning and a 24-hour sputum collection from the preceding 24hours of their appointment.

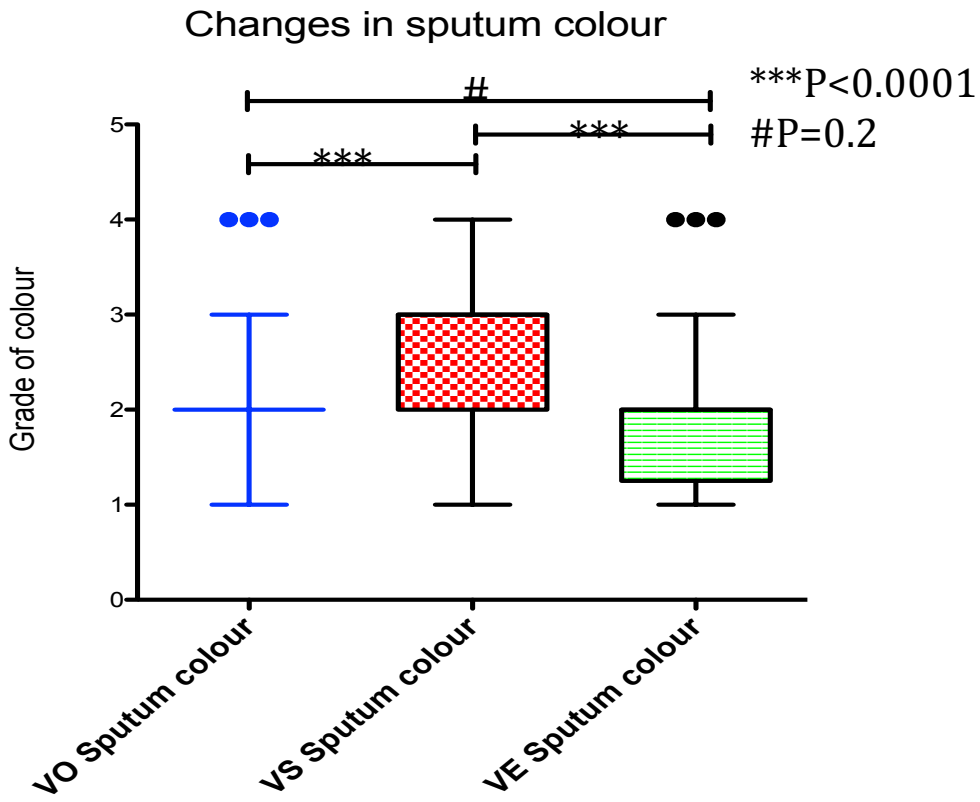
4.4.3.1 Sputum colour

Sputum was analysed for its colour and graded according to a standardised colour chart. The colour was awarded a number of 1 to 4 with 1 being awarded to clear or grey sputum and the number increasing with increasing purulence (see sputum purulence colour chart – 2= light yellow or light green, 3/4= dark yellow/green/brown colour).

89 patients provided a spontaneous sputum sample at baseline. 94 patients provided a spontaneous sputum sample at start of exacerbation and 84 at end of exacerbation. At baseline the median colour of spontaneous sputum was grade 2. This increased to a median colour of grade 3 at start of exacerbation which then returned to a baseline median value of grade 2 at the end of exacerbation.

There was a significant increase in sputum colour from baseline to start of exacerbation. This reduced back to baseline level at the end of exacerbation (P<0.0001) with no significant difference in sputum colour seen from baseline to end of exacerbation (P=0.2) – see figure 5.

Figure 5.

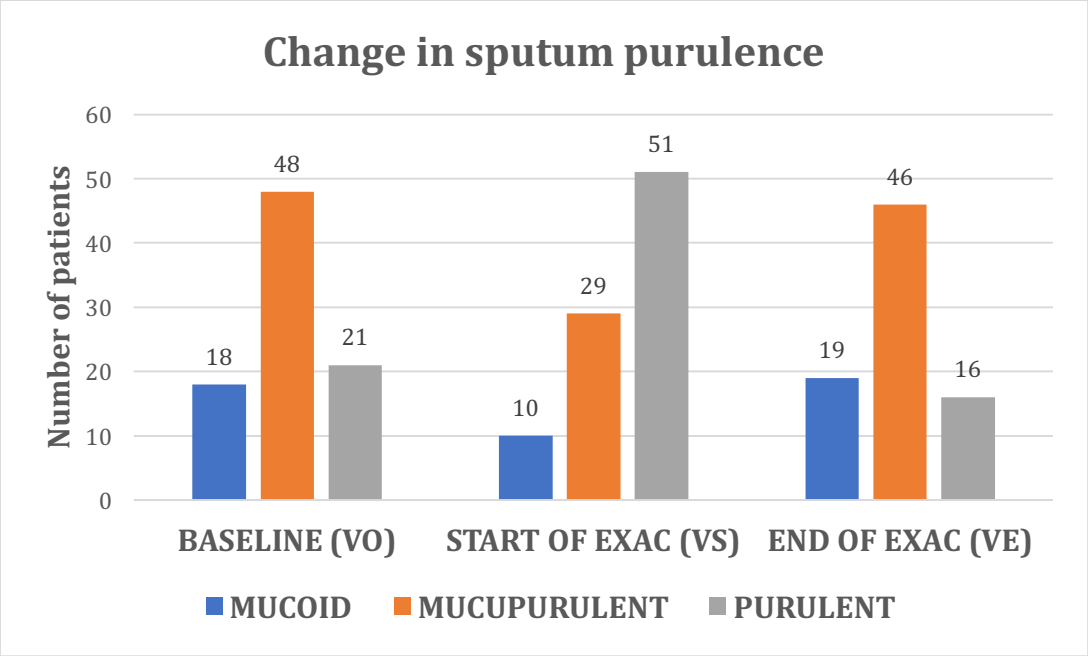


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in sputum colour from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, ***p<0.001, #p>0.05.

4.4.3.2 Sputum purulence

Sputum colour grade was divided into three groups with grade 1 classed as mucoid, grade 2 classed as mucopurulent and grades 3 and 4 classed as purulent. Mucopurulent was the predominant sputum grade at baseline. Sputum at the start of exacerbation were more purulent with less mucoid samples than baseline. At the end of exacerbation, the predominant grade was mucopurulent.

Figure 6.

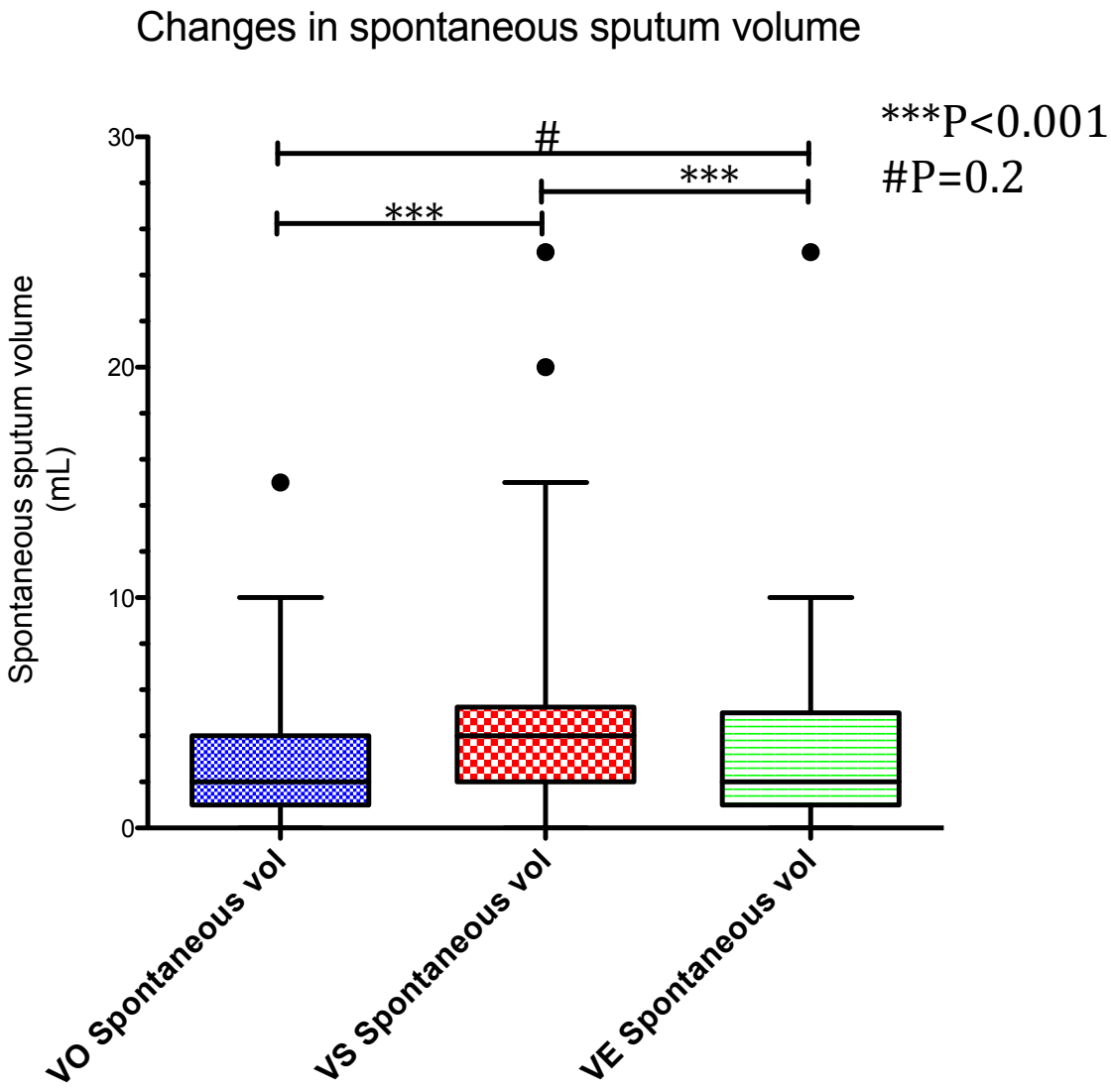


Bar chart to show the change in sputum purulence from baseline (VO) to start (VS) and end of exacerbation (VE).

4.4.3.3 Spontaneous sputum volume

Spontaneous sputum produced within 4hrs from waking on the morning of each visit was measured. Results are shown in figure 7. Spontaneous sputum production changed with exacerbation. There was a median of 2mls produced by patients at baseline. This increased to 4mls at start of exacerbation visits ($P<0.0001$) and reduced back to 2mls at end of exacerbation ($P<0.001$). There was no difference in the amount of spontaneous sputum produced between baseline and end of exacerbation.

Figure 7.

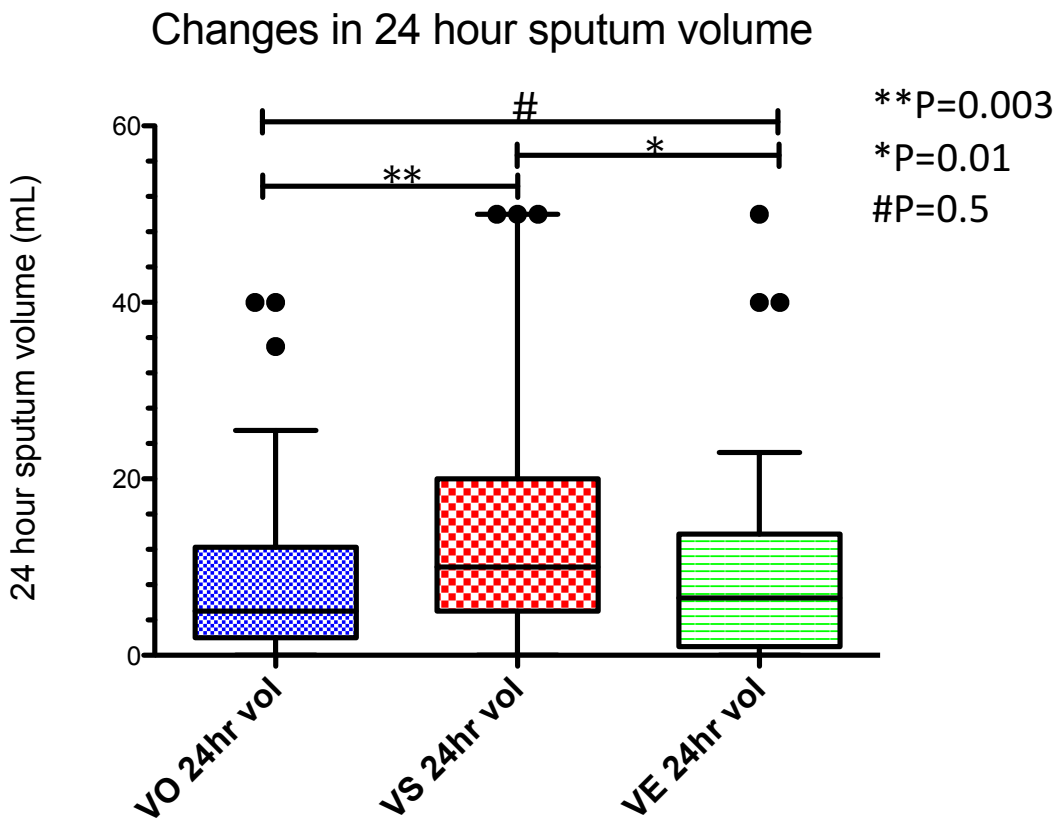


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in spontaneous sputum volume from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, ***p<0.001, #p>0.05.

4.4.3.4 24-hour sputum volume

Patients were asked to collect all sputum expectorated in a collection pot over a period of 24-hours prior to each clinic visit. This is particularly arduous for patients and at times the clinic visit (especially start of exacerbation visit) was arranged at short notice. Therefore 78 patients provided a 24-hour sample at baseline, 59 at start of exacerbation and 76 at end of exacerbation. Results are shown in figure 8.

Figure 8.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in 24hr sputum volume from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, *p<0.05, **P<0.01, #p>0.05.

24-hour sputum volume increased from 5mls at baseline to 10mls at start of exacerbation (P=0.003). It significantly reduced with treatment to 6.5mls at the end of exacerbation (P=0.001). There was no difference in 24-hour sputum production between baseline and end of exacerbation.

4.4.4 Medical research council (MRC) breathlessness score

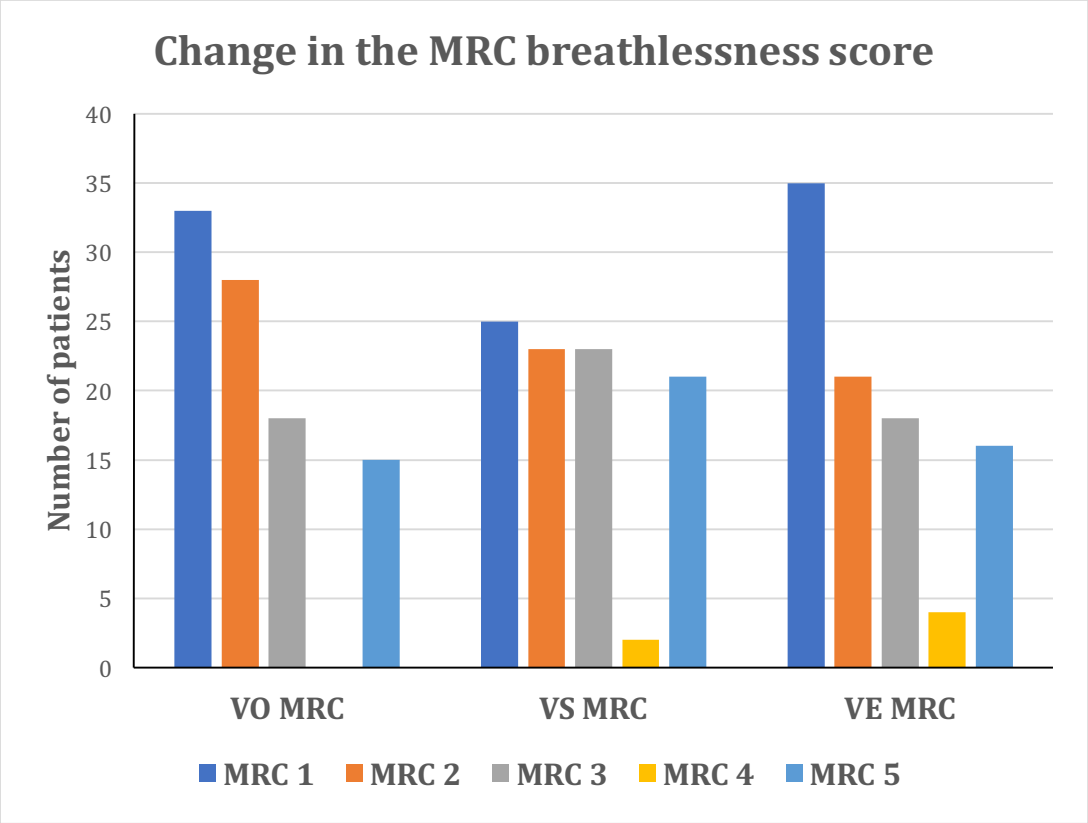
Patients with bronchiectasis often report breathlessness as a symptom. The MRC breathlessness score is a scale of 1-5 based on the degree of breathlessness- see below (Bestall *et al*, 1999).

MRC BREATHLESS SCORE

1	Not troubled by breathlessness, except on strenuous exercise
2	Breathless when hurrying on the level or walking up an incline
3	Walks slower than most on the level, stops after walking 1 mile or 15mins
4	Stops for breath after 100yards or few minutes walking on level ground
5	Breathless when dressing/undressing, unable to leave the house

The range of MRC scores appointed at each visit are depicted in figure 9. The most commonly occurring score at baseline is MRC score 1. There is an increase in the number of patients reporting MRC scores of 3, 4 and 5 at start of exacerbation. The pattern of MRC scores at end of exacerbation are similar to baseline scores.

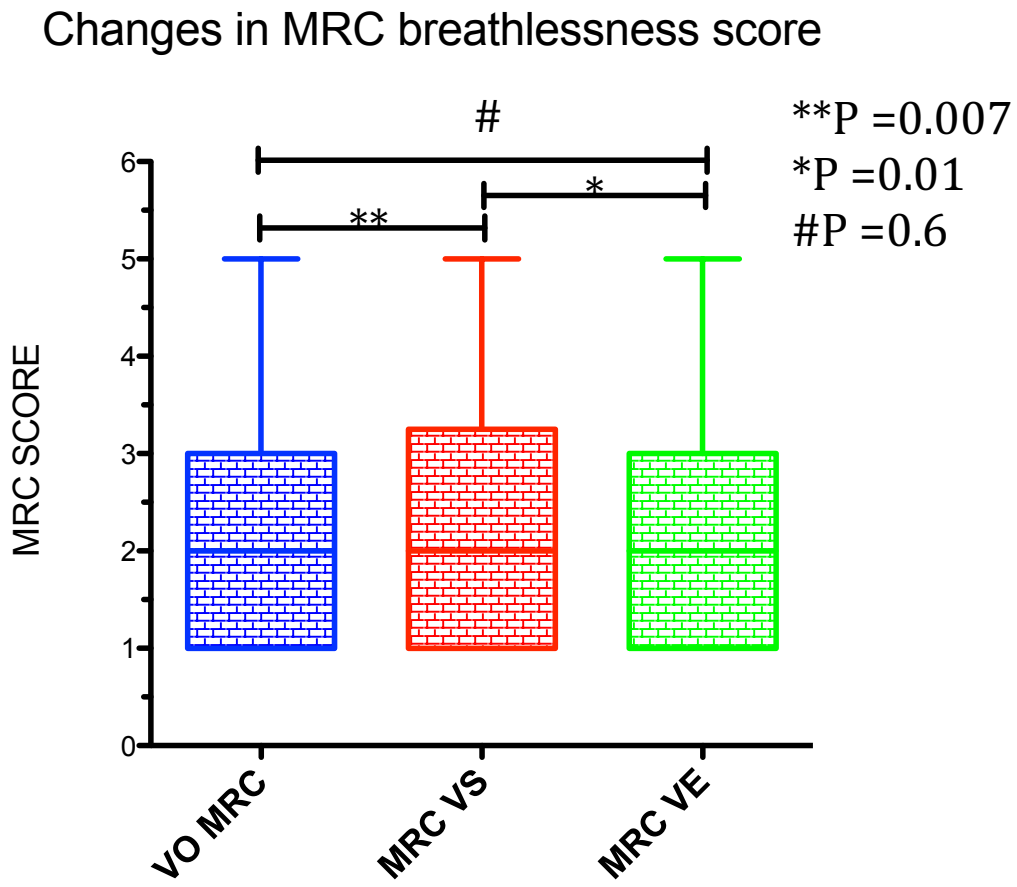
Figure 9.



Barchart to show the pattern of Medical Research Council Dyspnoea scores (MRC) levels 1-5 at different visits VO: baseline, VS: start of exacerbation, VE: end of exacerbation with increasing severity from level 1 to 5.

Paired statistical analysis of the MRC scores at different visits is shown in figure 10. The median MRC score at baseline, start of exacerbation and end of exacerbation was 2. The mean value increased from 2.3 at baseline to 2.7 at start of exacerbation (P=0.007). It then reduced to 2.4 (P=0.01) at end of exacerbation which was not significantly different from baseline (P=0.6).

Figure 10.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in MRC breathlessness score from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, *p<0.05, **P<0.01, #p>0.05.

4.4.4.2 Subanalysis of MRC score

MRC scores 1 and 2 represent relatively mild breathlessness and 3, 4 and 5 are for more symptomatic patients. Analysis using chi squared statistical test was performed to see if patients' breathlessness increased when unwell. There was an increase in

more symptomatic breathlessness at the start of exacerbation when compared with baseline scores although just failed to reach conventional statistical significance. There was no difference between start and end of exacerbation and no difference between baseline and end of exacerbation (Tables 9a, 9b & 9c).

Table 9a.

	MRC 1-2	MRC 3-5	
VO	61	33	
VS	48	46	**P=0.054**

Table 9b.

	MRC 1-2	MRC 3-5	
VS	**48**	**46**	
VE	**56**	**38**	**P= 0.2**

Table 9c.

	MRC 1-2	MRC 3-5	
VO	**61**	**33**	
VE	**56**	**38**	**P= 0.5**

Chi squared results of change in MRC results from 9a: stable (VO) to start (VS), 9b: start to end of exacerbation (VE), 9c: stable to end exacerbation.

4.4.5 Quality of life

4.4.5.1 The Leicester cough questionnaire

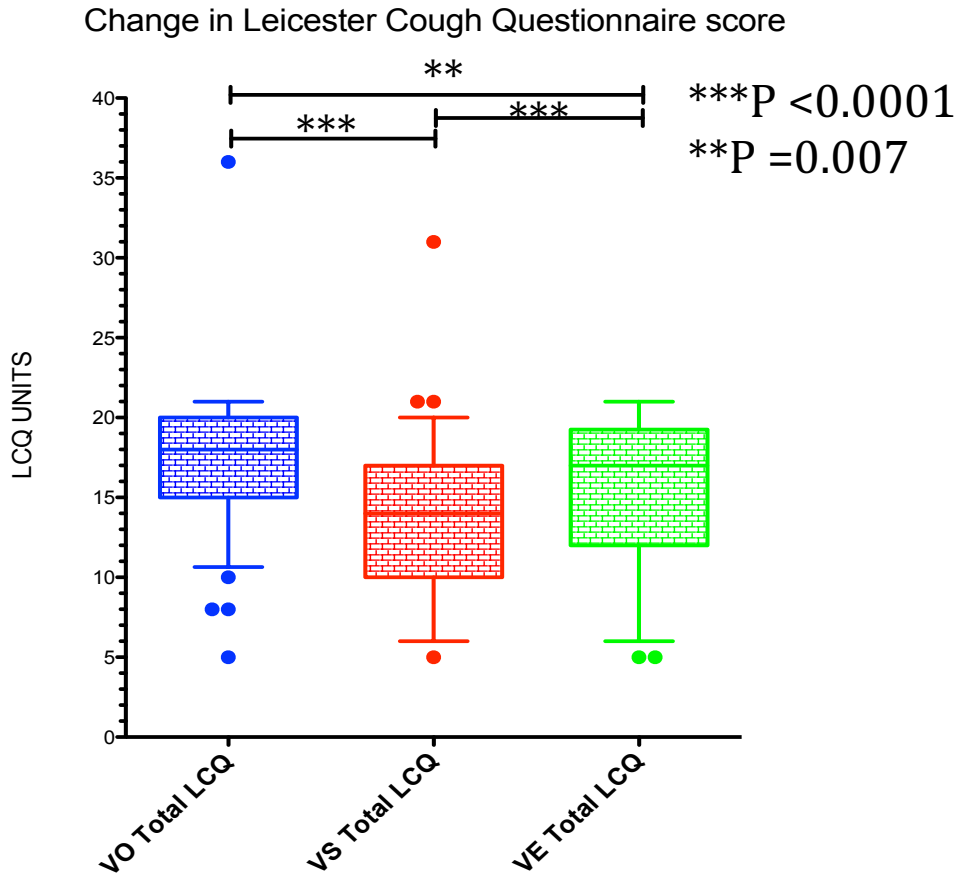
The Leicester cough questionnaire (LCQ) is a validated questionnaire shown to assess the impact of cough on quality of life in patients with bronchiectasis. The LCQ gives a score that range from 3 to 21. The lower the score the poorer the quality of life. All patients were required to complete a questionnaire at each visit. Results are shown in table 10 and figure 11.

Table 10.

	VO Total LCQ	VS Total LCQ	VE Total LCQ
Number of values	92	91	94
25% Percentile	15.00	10.00	12.00
Median	18.00	14.00	17.00
75% Percentile	20.00	17.00	19.25

Table to show the change in Leicester Cough Questionnaire (LCQ) Units when stable (VO) and with an exacerbation (VS and VE).

Figure 11.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Leicester Cough Questionnaire (LCQ) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, **p<0.01, ***P<0.001.

There is a significant decrease in LCQ from baseline to start of exacerbation. There is a significant improvement in scores at the end of exacerbation visit post 14 day course of antibiotics. There remained a difference between baseline and end of exacerbation suggesting patients did not feel fully recovered. The minimum clinically important difference in score is 1.3 units. This would suggest that patients felt clinically worse at start of exacerbation and improved at the end of exacerbation.

4.4.5.2 The St George's Respiratory Questionnaire (SGRQ)

The St George's Respiratory Questionnaire assess quality of life and gives a score ranging from 0 to 100. The higher the score the poorer the quality of life. The minimum clinically important difference is 4 units. See table 11 and figure 12 for results.

Table 11.

	VO Total SGRQ	VS Total SGRQ	VE Total SGRQ
Number of values	92	91	94
25% Percentile	18.00	23.00	20.00
Median	29.50	34.00	34.00
75% Percentile	48.50	52.00	51.00

Table to show the change in the St George's Respiratory Questionnaire (SGRQ) Units from stable (VO) to start and end of exacerbation (VS and VE).

Figure 12.

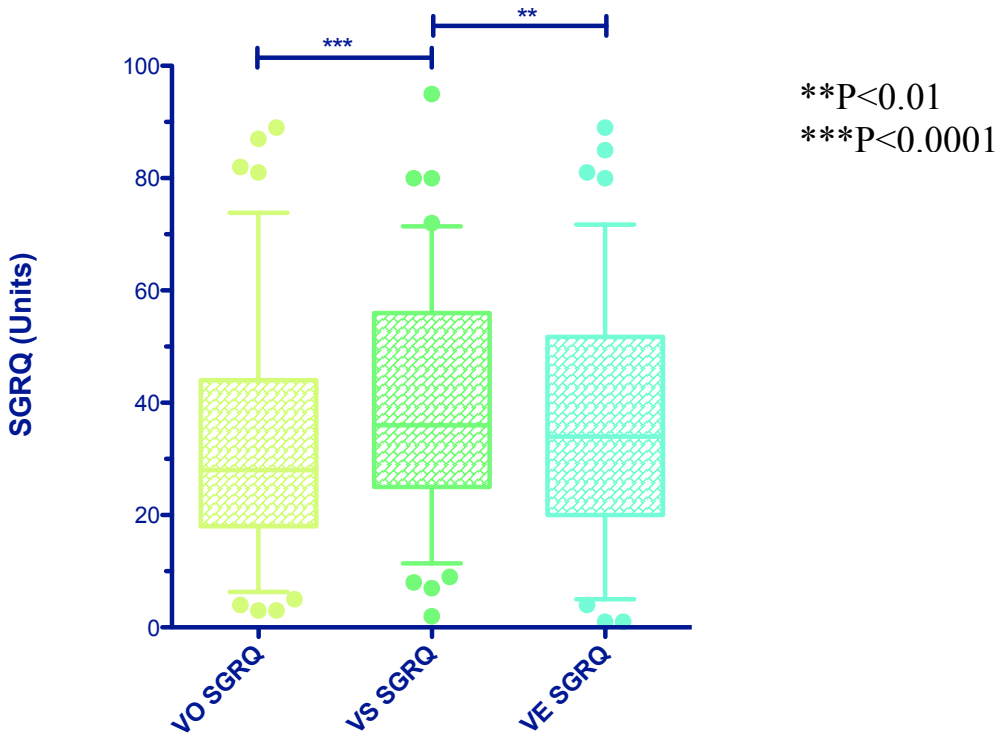


Figure to show the median value (IQR) St George's Respiratory Questionnaire (SGRQ) scores as baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Wilcoxon analysis **p<0.01, ***p<0.0001.

There is a significant worsening in SGRQ quality of life score at start of exacerbation (median 36 (25-36)) when compared with baseline (median 28 (18-44)) (P<0.00001). The median then reduced significantly with 14 days of antibiotics at end of exacerbation (median 34 (20-51.75)) (p=0.003).

4.4.6 Qualitative bacteriology

4.4.6.1 Bacterial culture results

Patients were asked to provide a spontaneous sputum sample produced within 4 hours of waking to perform quantitative and qualitative microbiological analysis. Out of 94 patients with exacerbations, 93 samples were analysed at baseline, 90 samples at start of exacerbation and 90 at the end of exacerbation.

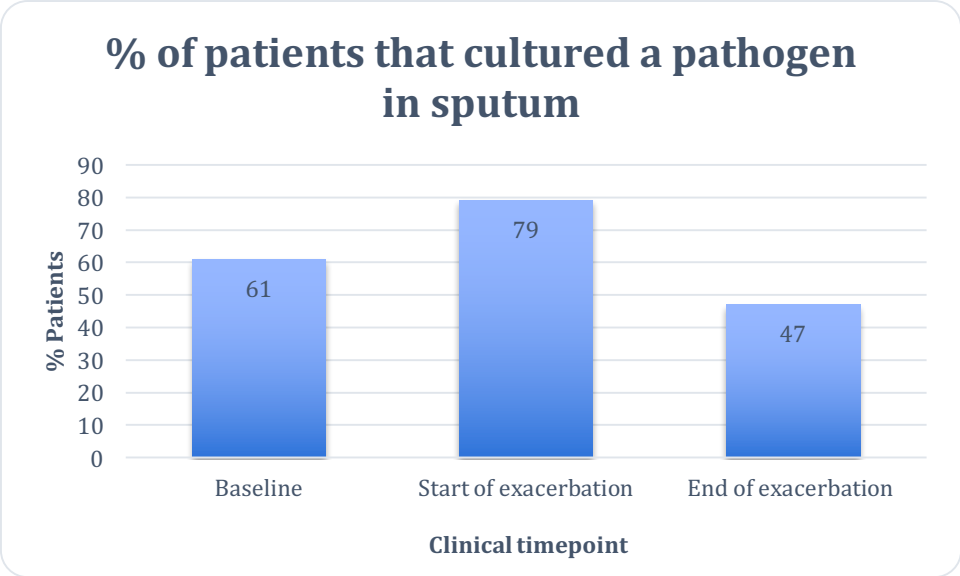
The predominant pathogens cultured included *Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Enterobacter, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Proteus mirabilis, Acinetobacter baumannii, Achromobacter xylosoxidans, Aeromonas hydrophila, citrobacter freundii, Stenotrophomonas maltophilia, mixed normal flora,* and occasionally there was no growth. Some patients were not expectorating sputum despite feeling unwell.

In order to analyse the data, the pathogens were placed into the four following groups:

- MNF/No growth - mixed normal flora, no growth and no sputum expectorated
- Potentially pathogenic microorganisms (PPM) - *Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus and Moraxella catarrhalis*
- *Pseudomonas aeruginosa* (PA)
- Other gram negative organisms - *Enterobacter, Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia, Serratia marcescens, Proteus mirabilis, Acinetobacter baumannii, Achromobacter xylosoxidans, citrobacter freundii and Aeromonas hydrophila*
- 61% of patients at baseline had sputum that cultured a pathogen. Of these, 25% cultured more than 1 microorganism.
- 79% of patients at start of exacerbation had sputum that cultured a pathogen. Of these, 30% cultured multiple pathogens.

47% of patients at the end of exacerbation had sputum that cultured a pathogen. Of these, 17% cultured multiple microorganisms –figure 13. The pathogens cultured at each visit are represented in pie-charts 14a, 14b and 14c.

Figure 13.



Increase in % of patients that cultured a pathogen at start of exacerbation.

Figure 14a.

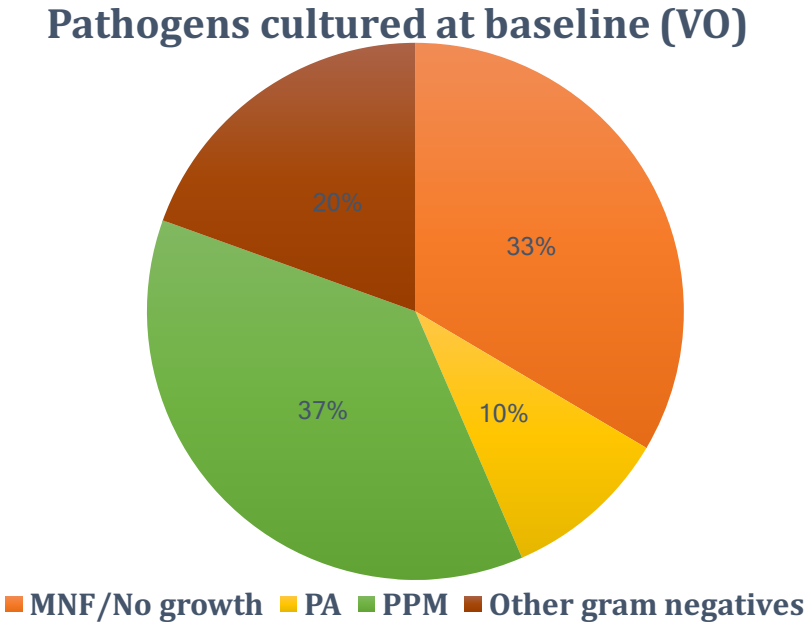


Figure 14b.

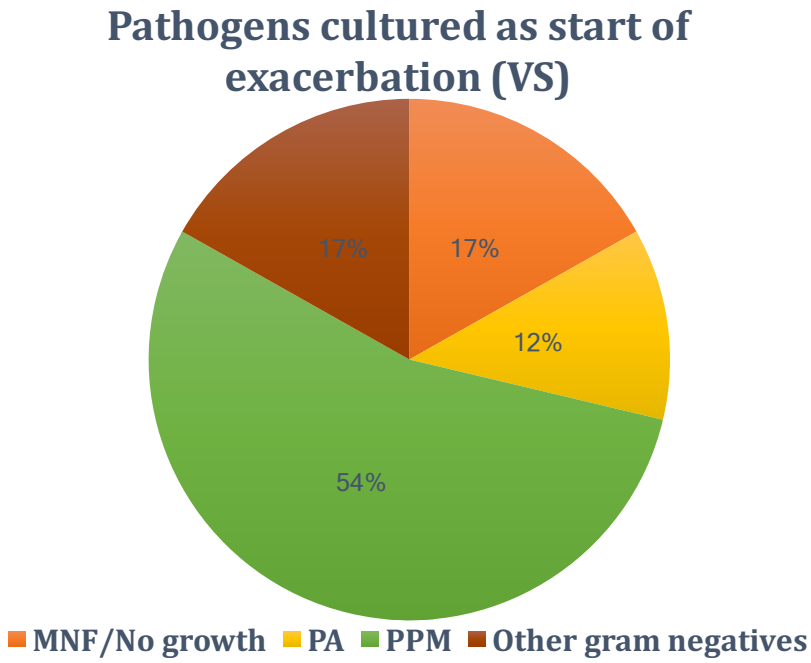
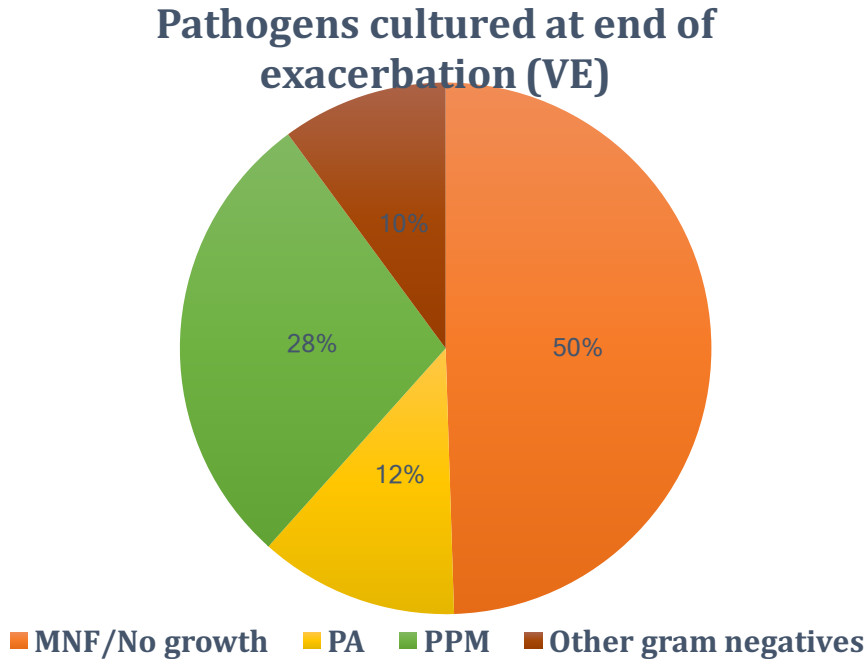


Figure 14c.



Piecharts to show the proportion of different microorganisms cultured 14a) Out of a total 108 results at baseline (VO), 14b) Total 113 results at start of exacerbation (VS) and 14c) A total of 97 results at end of exacerbation (VE). MNF: mixed normal flora, PA: *Pseudomonas aeruginosa*, PPM: other potentially pathogenic microorganisms.

There were significantly more patients with sputum samples that grew mixed normal flora or that had no sputum at baseline when compared with patients at the start of exacerbation. There were therefore more patients that had sputum that cultured a pathogen at the start of exacerbation than there was at baseline (P=0.009).

There was a significant increase in the number of samples that cultured mixed normal flora or had no sputum at the end of 14 days of antibiotics when compared with the start of exacerbation. There was therefore a significant reduction in the number of samples that cultured any pathogen (P=0.0001).

There was a similar trend when comparing sputum sample results at baseline and end of exacerbations with more sputum samples culturing pathogens at baseline and more samples confirming mixed normal flora or no growth at end of exacerbation (P=0.047).

4.4.6.2 Change in bacterial species cultured with exacerbation

Culture results from individual patients were analysed. There was a 57% overall change in the dominant pathogen cultured when comparing start of exacerbation with baseline culture results. Of these, a small proportion of results saw a switch to growing mixed normal flora (4%) and a much larger proportion (53%) saw a different pathogen being cultured (figure 15). At the end of exacerbation there was a much bigger switch to mixed normal flora (36%) when compared with start of exacerbation, as you might expect after a 14-day course of antibiotics and 23% switch to a different pathogen. There was a 34% switch of pathogen from baseline to end of exacerbation with a smaller 29% switch to mixed normal flora from baseline to end of exacerbation and this might be accounted for by a persistent change in bacteria isolated at start of exacerbation. The changes from VO to VS were statistically significant when compared with the change in bacterial culture to either MNF or different pathogen from VS to VE and from VO to VE (P<0.01).

Figure 15.

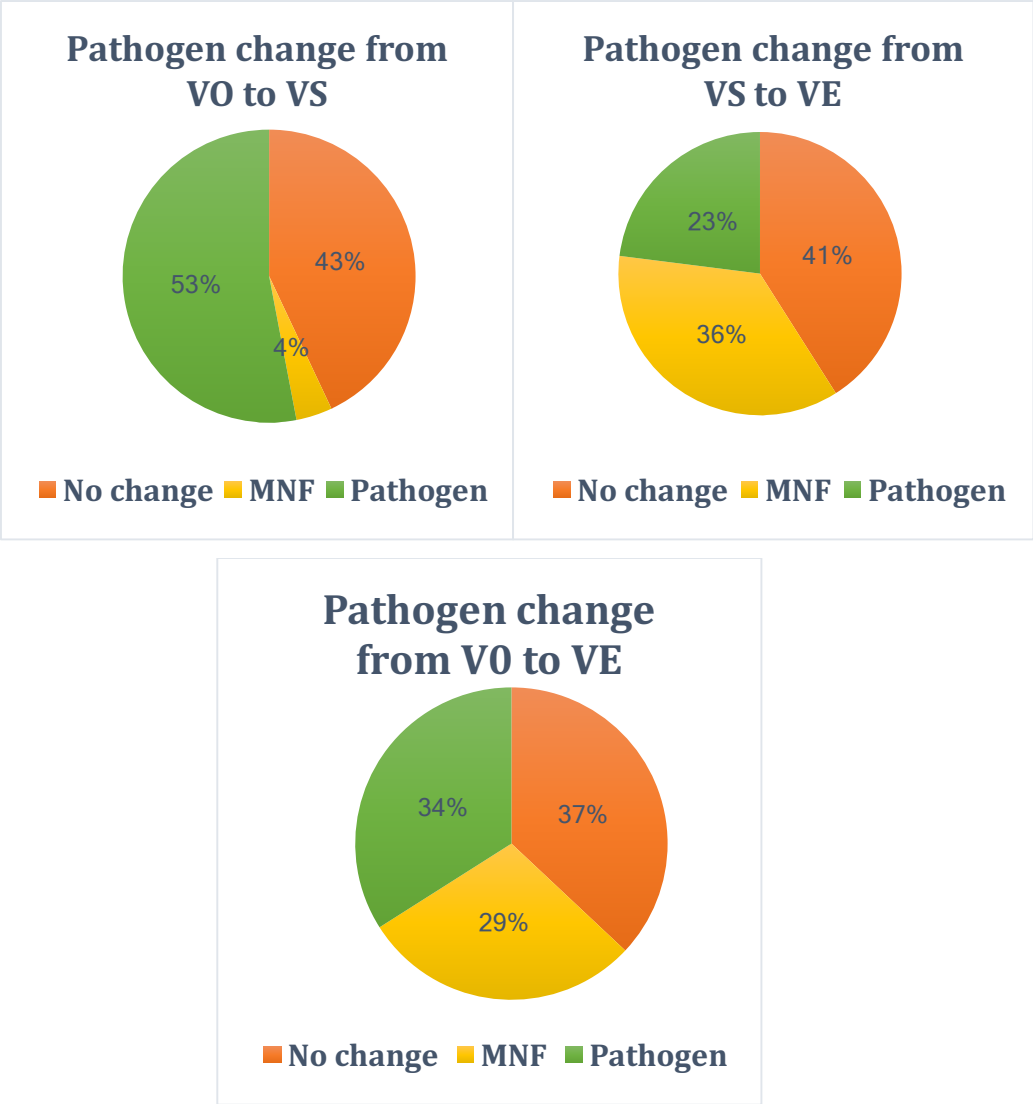
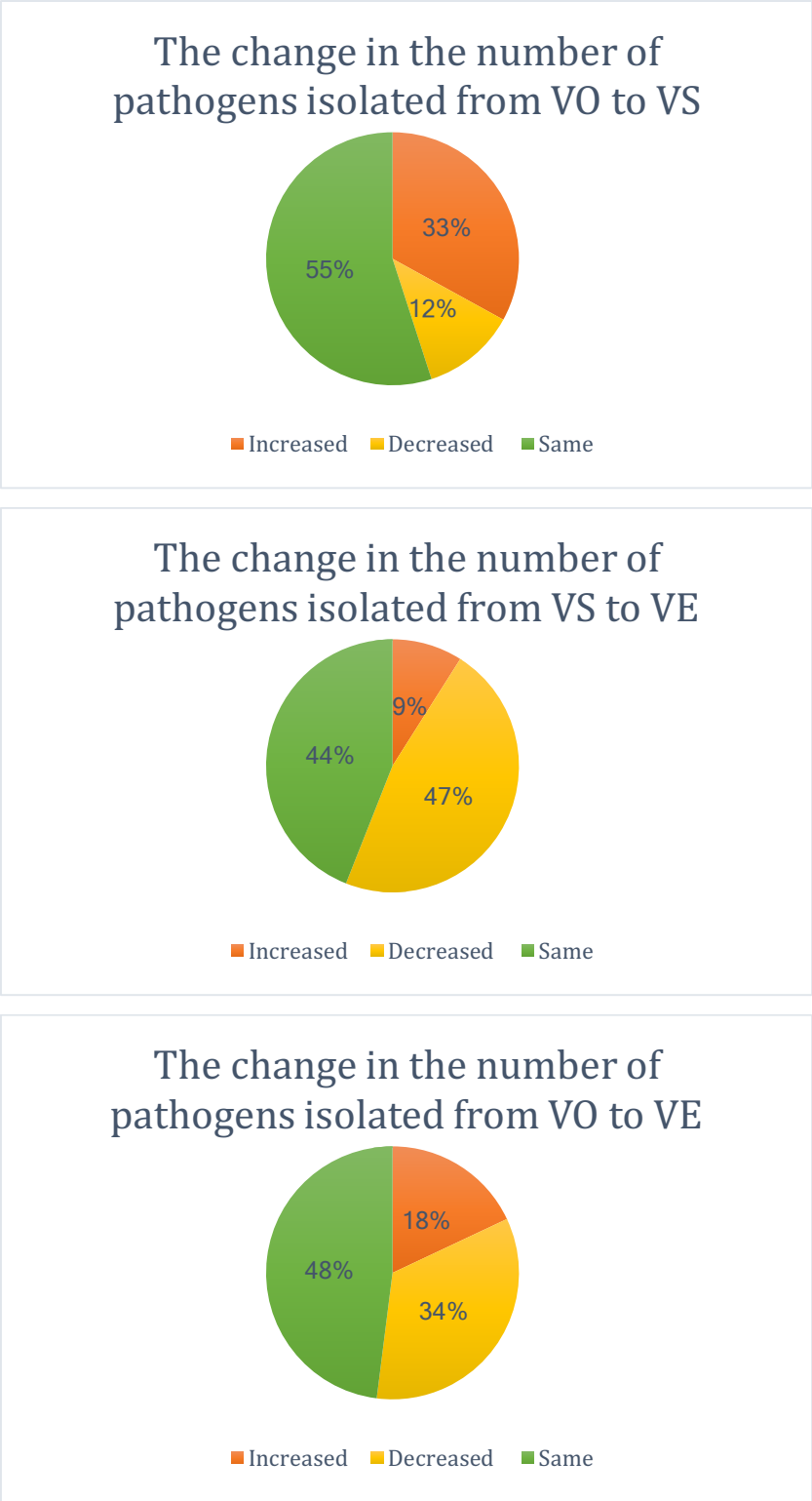


Figure to show the change in proportions of different microorganisms cultured at each time point baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). MNF: mixed normal flora.

4.4.6.3 Change in the number of pathogens isolated with exacerbation

There was a 33% increase in the number of culture results that isolated more pathogens at visit start than at baseline visit for the same patient (P<0.0001). In 55% of cases the number of isolates remained the same. At the end of exacerbation 47% of culture plates isolated fewer numbers of pathogens than when cultured at start of exacerbation for the same patient (P<0.0001). Results are outlined in figure 16 and table 12.

Figure 16.



Figures to show the change in numbers of pathogens cultured per patients from baseline (VO) to start of exacerbation (VS) *top*, start to end of exacerbation (VE) *middle* and from baseline to end of exacerbation *bottom*.

Table 12.

	Visit start (VS)	Visit end (VE)
Change in the number of pathogens cultured compared with VO	**33% of patients cultured more pathogens ↑**	**34% of patients cultured less pathogens ↓**
P value	**<0.0001**	**<0.0001**
Change in the number of pathogens cultured compared with VS		**47% of patients cultured less pathogens ↓**
P value		**<0.0001**

Table to show the change in the number of pathogens cultured per patient with exacerbation with chi squared results.

4.4.7 Quantitative microbiological analysis

4.4.7.1 Bacterial load

Sputum samples were diluted and plated out as per protocol. After 48 hours of incubation the plates were analysed and bacteria identified. Individual colony forming units (CFU) were counted to ascertain the bacterial load. Results are presented in table 13 and figure 17.

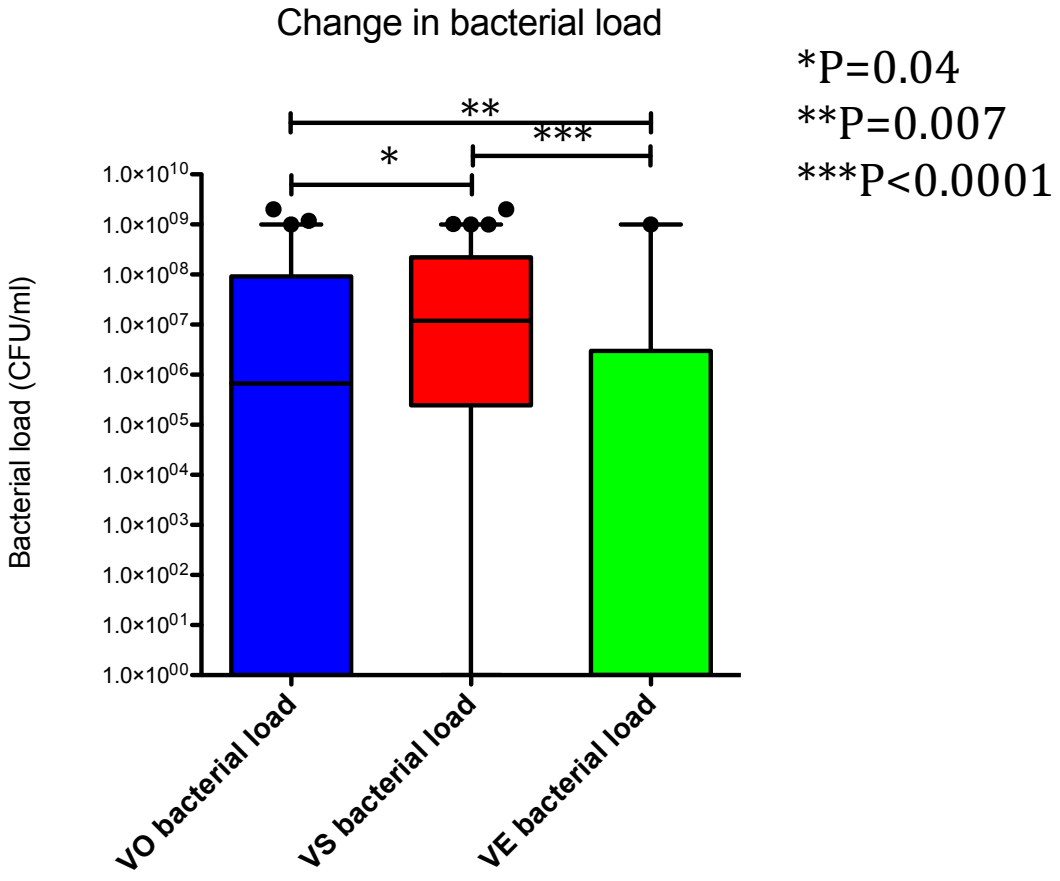
The median bacterial count is 6.7 x 10^5 CFU at baseline. This increases to 1.2 x 10^7 at start of exacerbation and returns to a median value of 0 CFU at the end of exacerbation visits.

Table 13.

	Bacterial load VO (CFU/ml)	Bacterial load VS (CFU/ml)	Bacterial load VE (CFU/ml)
Number of values	93	90	90
25% Percentile	0	1.8×10^5	0
Median	6.7×10^5	1.2×10^7	0
75% Percentile	9.22×10^7	2.213×10^8	3×10^6

Table to show the change in bacterial load (CFU/ml) from baseline (VO) to start (VS) and end of exacerbation (VE).

Figure 17.



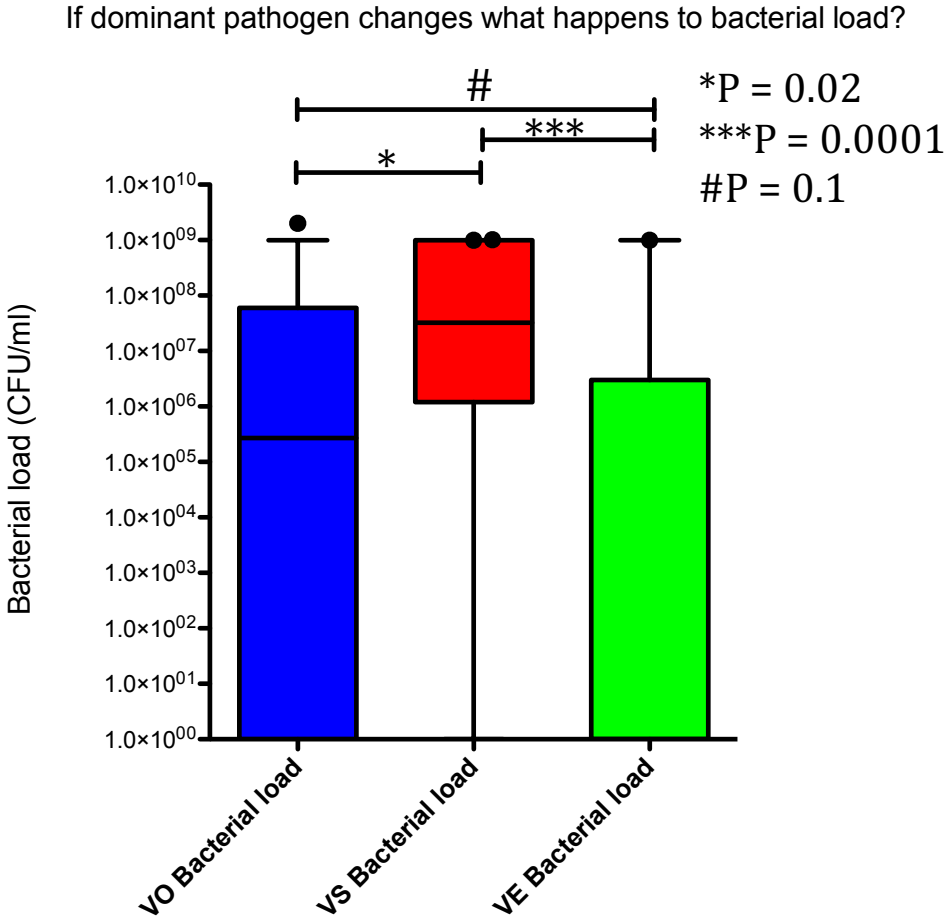
Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in bacterial load (CFU/ml) from baseline (VO) to start of exacerbation (VS) to end of exacerbation (VE). Significance, *p<0.05, **P<0.01, ***p<0.001.

There was a significant 1.5 log increase in bacterial load from baseline to start of exacerbation (P=0.04). The effect of 14 days of antibiotic treatment also significantly reduces the bacterial load (P<0.0001). Bacterial load is reduced below baseline level at the end of antibiotic treatment (P=0.007).

4.4.7.2 Bacterial load when the dominant pathogen changes

57% of patients had a different sputum pathogen cultured at start of exacerbation (VS) when compared with their baseline visit (VO) sputum results – see figure 14. The bacterial load was analysed in these patients – figure 18 and table 14.

Figure 18.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in bacterial load (CFU/ml) when sputum microbiology changes at exacerbation, from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, *p<0.05, ***p<0.001, #p>0.05.

Table 14.

	VO Bacterial load (CFU/ml)	VS Bacterial load (CFU/ml)	VE Bacterial load (CFU/ml)
Number of values	51	51	49
25% Percentile	0	1.2×10^6	0
Median	2.7×10^5	3.2×10^7	0
75% Percentile	6.0×10^7	1.0×10^9	3.6×10^6

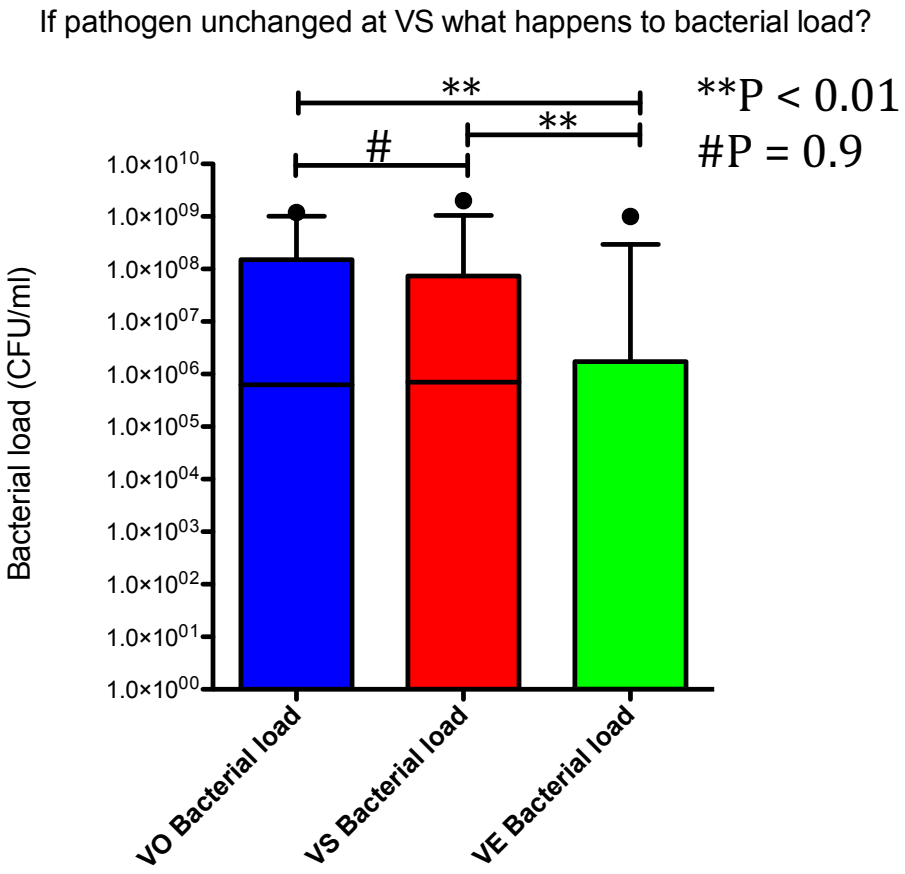
Table to show the change in bacterial load in exacerbation when sputum microbiology changes.

There is a 1.5 log increase in bacterial load when a change in pathogen is detected on sputum culture microbiology. The bacterial load significantly increased from a median value of 2.7×10^5 CFU/ml to 3.2×10^7 CFU/ml on exacerbation (P=0.02). This value reduces to 0 CFU/ml at the end of exacerbation and after 14days of antibiotics (P=0.0001). There is no difference between baseline bacterial load and that at the end of exacerbation.

4.4.7.3 Bacterial load when the dominant pathogen is unchanged.

In 43% of patients that experienced an exacerbation, the sputum microbiology did not differ from baseline to start of exacerbation. The changes in bacterial load was analysed in this subset of patients and presented in figure 19 and table 15.

Figure 19.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in bacterial load (CFU/ml) when sputum bacteriology is unchanged at exacerbation from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, **p<0.01, #p>0.05.

Table 15.

	VO Bacterial load (CFU/ml)	VS Bacterial load (CFU/ml)	VE Bacterial load (CFU/ml)
Number of values	38	38	38
25% Percentile	0.0	0.0	0.0
Median	6.3 x10^5	7.0 x10^5	0.0
75% Percentile	1.5 x10^8	7.4 x10^7	1.7 x10^6

Change in bacterial load (CFU/ml) from baseline (VO), start (VS) to end of exacerbation (VE) when sputum bacteriology is unchanged at exacerbation.

There is no significant change in bacterial load in this subset of patients with a median value of 6.3 $x10^5$ CFU/ml on baseline and 7.0 x 10^5 CFU/ml at start of exacerbation. There is significant reduction in bacterial load after 14 days of antibiotics at the end of exacerbation when compared with baseline levels (P=0.007) and start of exacerbation levels (P=0.002).

4.4.7.4 Change in dominant pathogen associated with a ≥1log rise in bacterial load?

A change in dominant pathogen is associated with an increase in bacterial load from baseline to start of exacerbation in these patients. To further assess the rise in bacterial load, results were analysed to see if there was a significant difference between the number of patients that had a 1 or more log rise in bacterial load depending on whether the dominant pathogen changed. Results are outlined below:

Table 16.

	Dominant Pathogen changed	Dominant pathogen unchanged
≥1Log rise in bacterial CFU/ml	29	8
≤1Log rise in bacterial CFU/ml	22	30

Table to show the significance of ≥1Log rise in bacterial load when dominant pathogen changes.

There was a significantly higher proportion of patients that had a 1 or more log rise in bacterial load if the dominant pathogen on sputum culture had changed at start of exacerbation then if the dominant pathogen had not changed (P=0.0007).

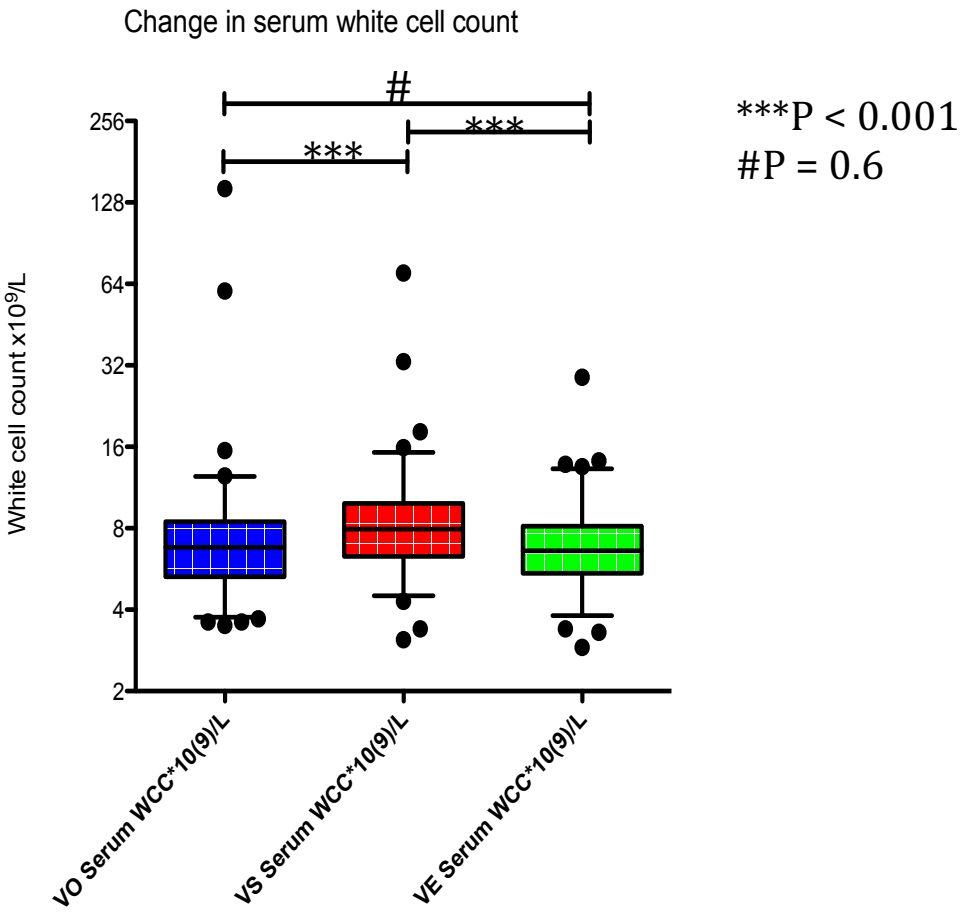
4.4.8 Blood inflammatory markers

Blood was taken from each patient at every visit. Bloods were analysed for full blood count, urea and electrolytes, liver function tests and inflammatory markers erythrocyte sedimentation rate and C-reactive protein. Important markers of infection and inflammation are shown in the figures below and table 17.

4.4.8.1 White cell count (WCC)

There was a significant increase in WCC from baseline (VO) to start of exacerbation (VS). There was a significant reduction in white cell count after 14 days therapy of antibiotics with no significant change between baseline and end of exacerbation which could imply the white cell count had returned to baseline (Figure 20).

Figure 20.

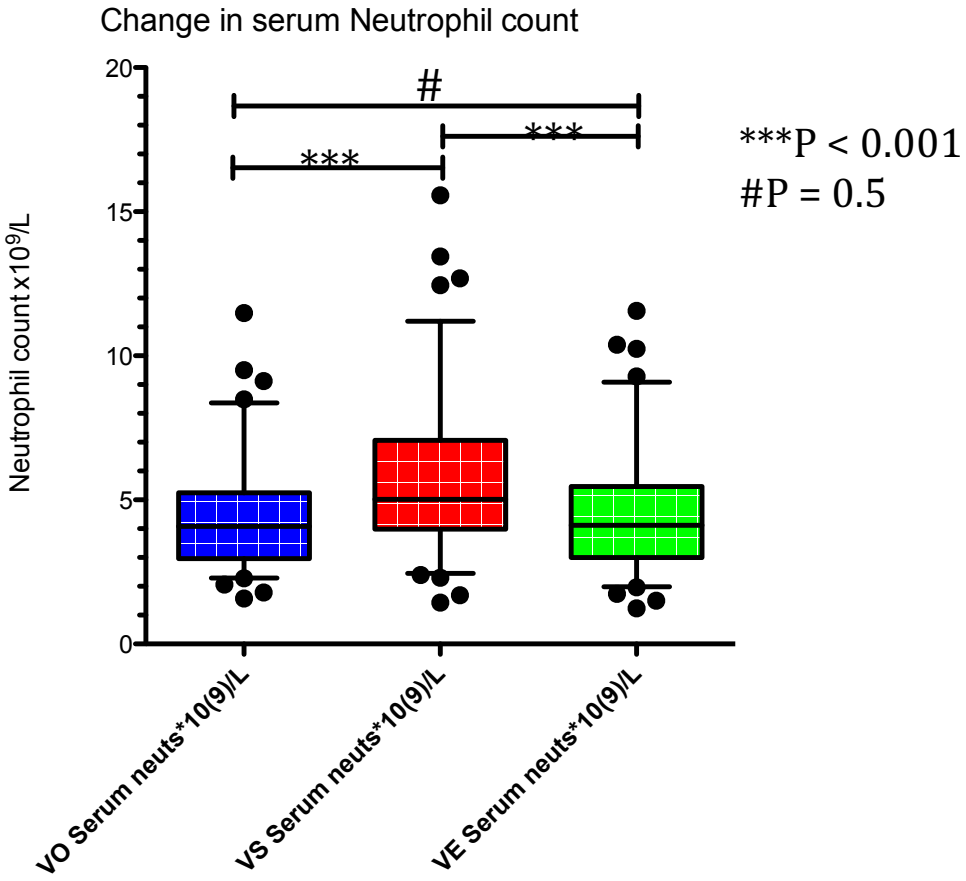


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in White Cell Count (WCC) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, ***p<0.001, #p>0.05.

4.4.8.2 Neutrophil count

There was a significant increase in neutrophil count at start of exacerbation which decreased again after 14 days of treatment. There was no difference in neutrophil count between baseline and end of exacerbation (Figure 21).

Figure 21.

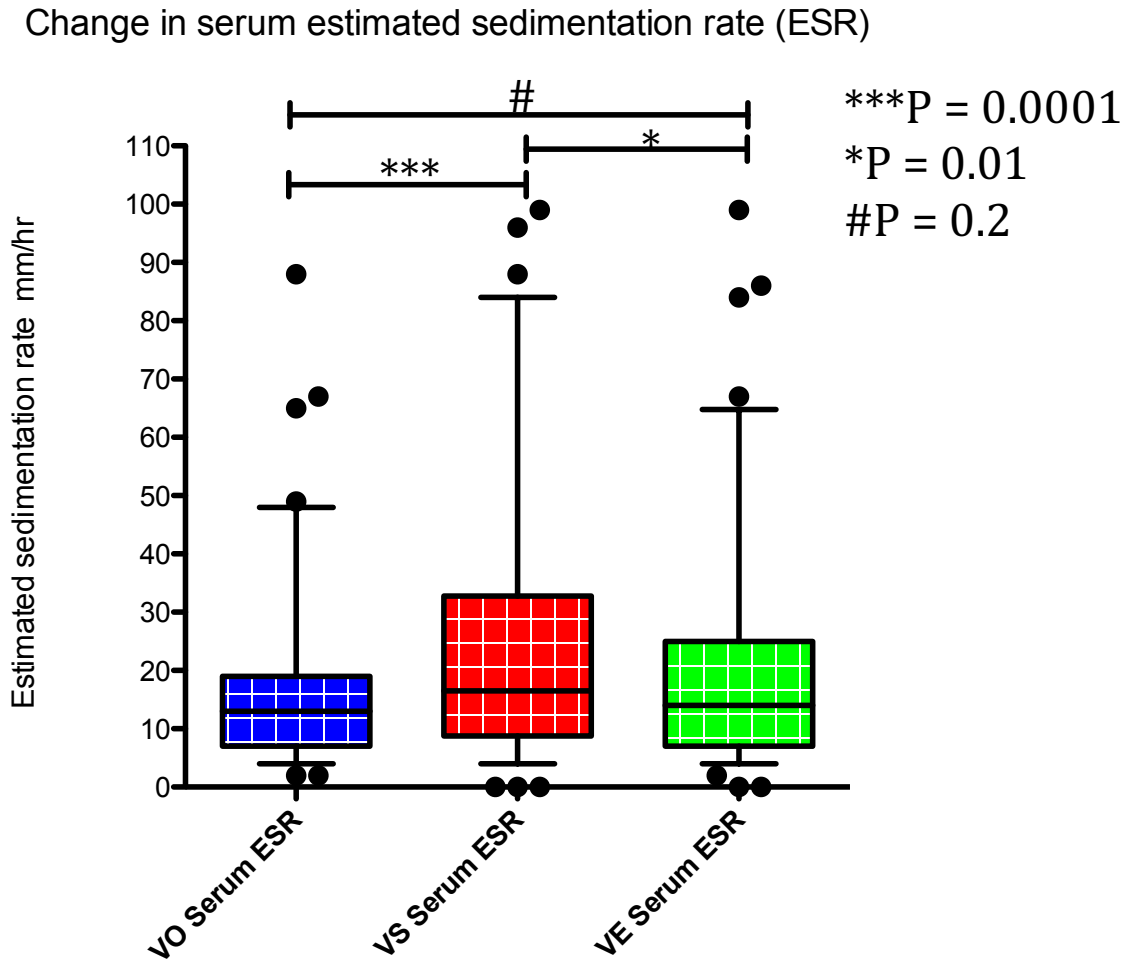


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Neutrophil count (neuts) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, ***p<0.001, #p>0.05.

4.4.8.3 Erythrocyte sedimentation rate (ESR)

There was a significant increase in ESR from baseline (VO) to start of exacerbation (VS). There was a significant reduction from VS to end of exacerbation (VE). There was no significant change between baseline and end of exacerbation (Figure 22).

Figure 22.

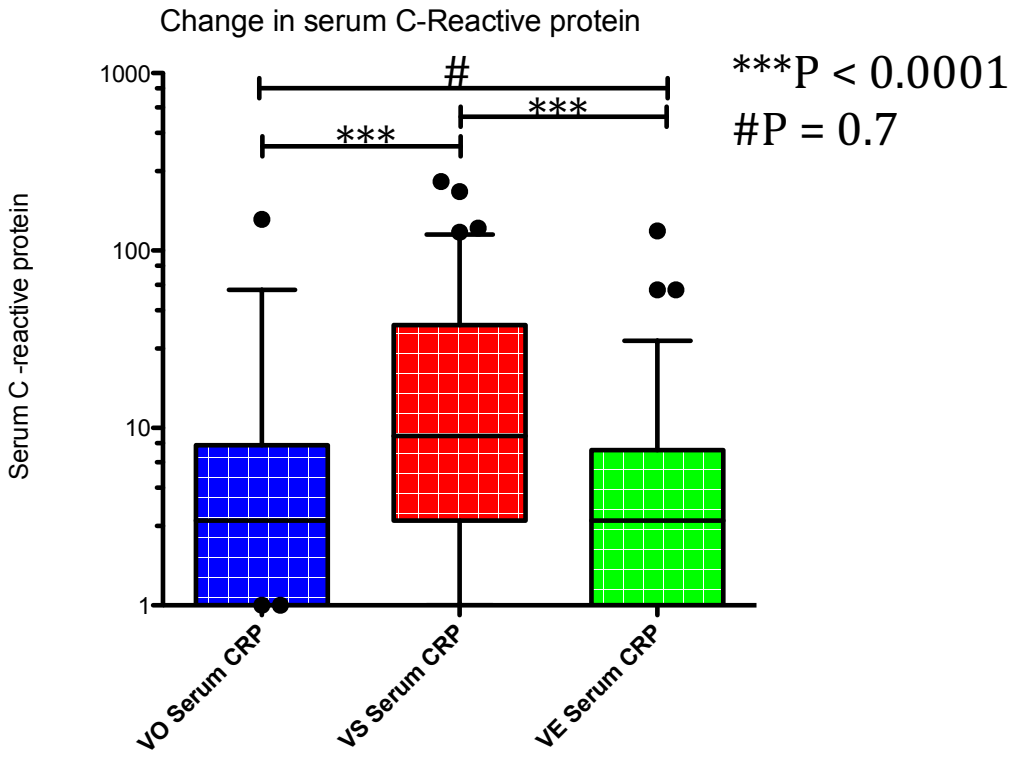


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Erythrocyte sedimentation rate (ESR) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, ***p<0.001, *p<0.05, #p>0.05.

4.4.8.4 C-reactive protein (CRP)

There was a significant increase in CRP from baseline to start of exacerbation, rising from a median 3.0 to 9.0 mg/L. There was a significant reduction from VS to end of exacerbation (VE). There was no significant change between baseline and end of exacerbation with median of 3.0 and 3.0mg/L respectively (Figure 23).

Figure 23.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in C-reactive protein (CRP) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, ***p<0.001, #p>0.05.

Table 17.

INFLAMMATORY MARKER	VALUE	BASELINE (VO)	VISIT START (VS)	VISIT END (VE)
White cell count x 10^9/L	25% Percentile	5.3	6.3	5.5
	Median	6.8	8.0	6.6
	75% Percentile	8.5	9.9	8.2
Neutrophil count x 10^9/L	25% Percentile	3.0	4.0	3.0
	Median	4.1	5.0	4.1
	75% Percentile	5.3	7.1	5.5
Erythrocyte sedimentation rate mm/hr	25% Percentile	7.0	8.8	7.0
	Median	13.0	16.5	14.0
	75% Percentile	19.0	32.8	25.0
C- reactive protein mg/L	25% Percentile	1.0	3.0	1.0
	Median	3.0	9.0	3.0
	75% Percentile	8.0	38.0	7.5

Table to show the change in blood inflammatory markers from baseline (VO) to start of exacerbation (VS) and end of exacerbation (VE).

There was a significant increase in all the infection and inflammatory markers analysed (WCC, Neutrophils, ESR & CRP). All markers significantly improved with treatment. There was no significant change between pre-infection and post treatment markers, suggesting levels had gone back to baseline.

4.4.9 Sputum inflammatory markers

4.4.9.1 Myeloperoxidase

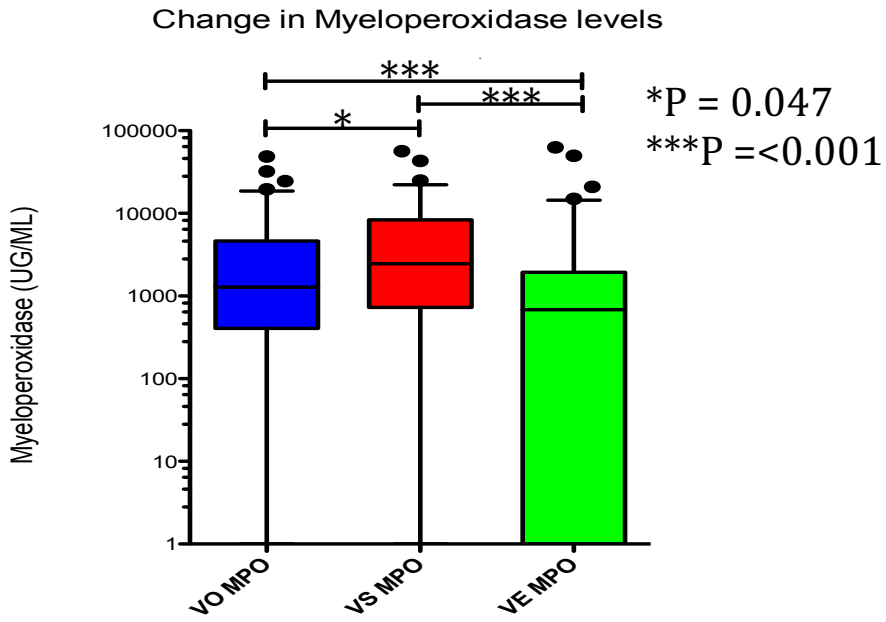
Myeloperoxidase levels significantly increased from baseline to start of exacerbation. Levels reduced after 14 days of antibiotics to a level significantly lower than baseline levels – see figure 24 and table 18 for median (IQR) values.

Table 18.

	VO MPO (UG/ML)	VS MPO (UG/ML)	VE MPO (UG/ML)
Number of values	85	77	84
25% Percentile	405.0	726.5	0.0
Median	1279	2462	682.5
75% Percentile	4626	8356	1935

Table to show the change in myeloperoxidase (MPO) levels from baseline (VO) to start (VS) and end of exacerbation (VE).

Figure 24.

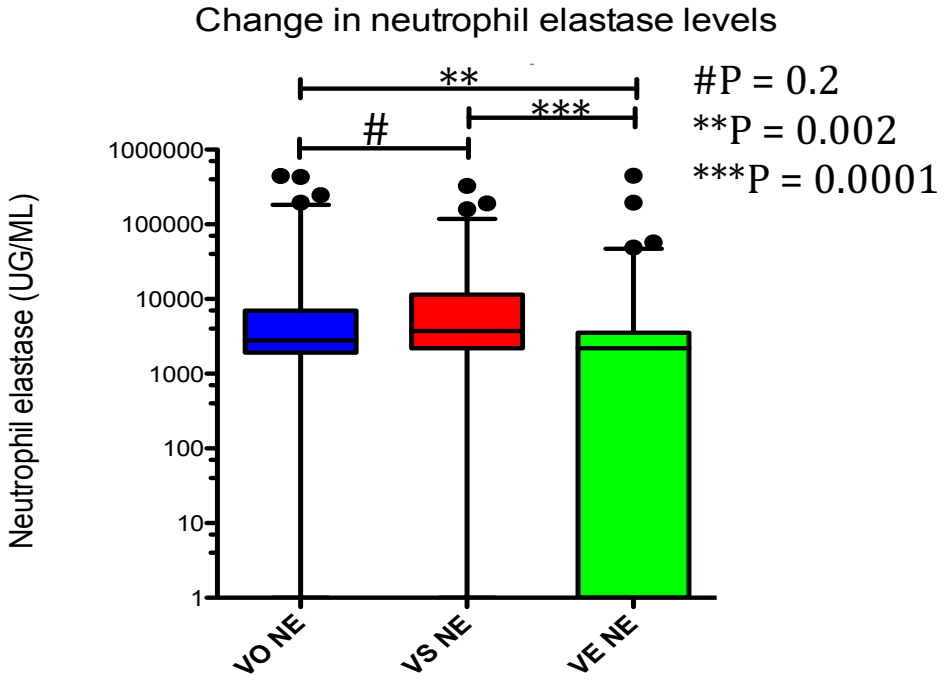


Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Myeloperoxidase (MPO) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, *p<0.05, ***p<0.001.

4.4.9.2 Neutrophil elastase (NE)

Neutrophil elastase levels increased from a median level of 2788µg/ml at baseline to 3728µg/ml but this increase was not statistically significant. Results significantly reduced after 14 days of antibiotics to 2200µg/ml, significantly lower than pre-infection baseline levels (figure 25, table 19).

Figure 25.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Neutrophil elastase (NE) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, **p<0.01, ***p<0.001, #p>0.05.

Table 19.

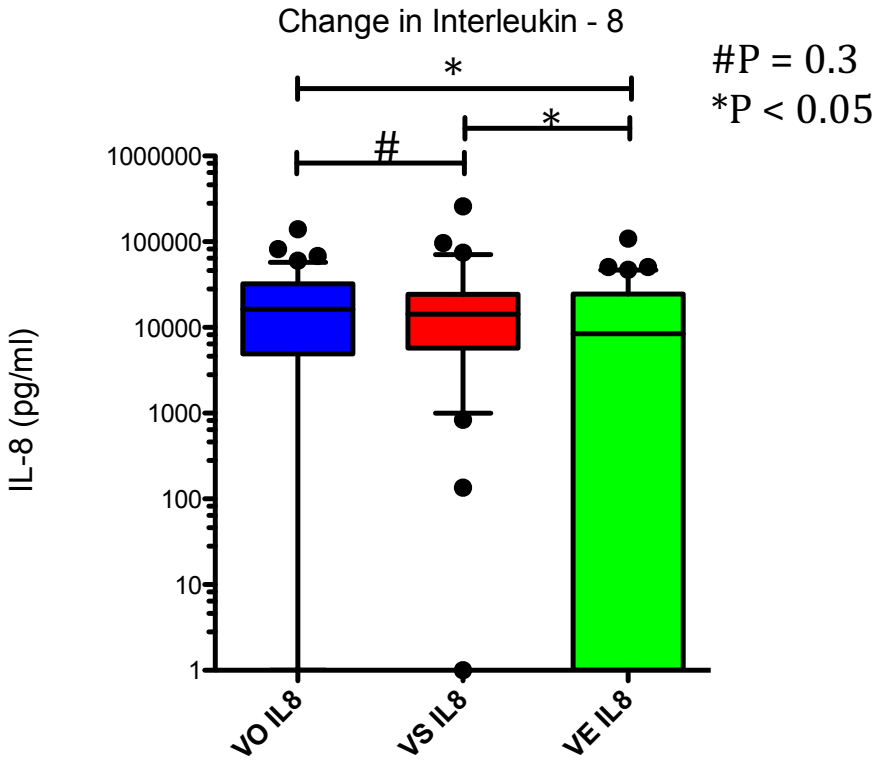
	VO NE (UG/ML)	VS NE (UG/ML)	VE NE (UG/ML)
Number of values	87	78	85
25% Percentile	1913	2194	0.0
Median	2788	3728	2200
75% Percentile	6983	11429	3532

Table to show the change in neutrophil elastase (NE) levels from baseline (VO), start (VS) and end of exacerbation (VE).

4.4.9.3 Interleukin 8 (IL-8)

There was no significant change in IL-8 level from baseline to start of exacerbation. There was a significant reduction in IL-8 after 14 days of antibiotics when compared with start of exacerbation and pre-infection baseline levels (figure 26, table 20).

Figure 26.



Boxplot (median, IQR with whiskers 5-95% percentile) to show the change in Interleukin-8 (IL-8) from baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Significance, *p<0.05, #p>0.05.

Table 20.

	VO IL8 Pg/ml	VS IL8 Pg/ml	VE IL8 Pg/ml
Number of values	88	76	80
25% Percentile	4903	5753	0.0
Median	16308	14271	8416
75% Percentile	32361	24408	24517

Table to show the change in Interleukin-8 (IL-8) levels from baseline (VO) to start and end of exacerbation (VS and VE).

4.5 Sub-analysis

A change in the dominant pathogen cultured caused a significant rise in bacterial load of more than 1 log CFU. A sub-analysis was carried out to see whether a 1 or more log rise in bacterial CFU was significant in causing increased inflammation or worsening of clinical parameters compared with a bacterial load rise of less than 1 Log unit CFU.

4.5.1 Clinical parameters

Patient results were split into two groups depending on the bacterial load change from baseline to start of exacerbation. The two groups (≥1log increase in bacterial load and <1log rise) were compared to see if there was any difference in the change of clinical parameters with infection (table 21).

Table 21.

VO⇒VS	≥1 LOG ↑ BL	<1 LOG ↑ BL	P VALUE
FEV_1 (L)	-0.06 (-0.2 – 0.045)	-0.10 (-0.168 – 0.01)	0.9
>200mls or >12%	14/38	14/52	**0.05**
>5%	20/38	24/52	0.67
>10%	16/38	11/52	0.04*
>15%	9/38	8/52	0.42
>20%	4/38	7/52	0.75
% FEV_1 (%)	4.2 (¯1.9 – 13.8)	3.6 (¯0.77 – 7.77)	0.7
>5%	18/38	22/52	0.67
>10%	16/38	11/52	0.04*
>15%	9/38	8/52	0.42
>20%	4/38	6/52	1.0
FVC (L)	-0.185 (-0.44 – 0.01)	-0.21 (¯0.43 - 0)	0.91
>200mls or >12%	19/38	29/52	0.67
>5%	22/38	31/52	1.0
>10%	14/38	19/52	1.0
>15%	12/38	9/52	0.13
>20%	6/38	7/52	0.7
% FVC (%)	-4.355 (¯0.96 – 17.7)	-6.155 (¯1.21 – 12.83)	0.75
>5%	18/38	30/52	0.40
>10%	13/38	18/52	1.0
>15%	12/38	10/52	0.22
>20%	6/38	7/52	0.7
LCQ (Units)	-4.0 (¯6.75 - ¯1)	-3.0 (¯5.75 - ¯1)	0.8
LCQ ≥1.3unit ↓	28/38	35/52	0.64
SGRQ (Units)	2.0 (¯3 – 14.5)	3.0 (¯5 - 13)	0.6
SGRQ ≥4units ↑	17/38	24/52	1.0
24hr sputum vol. (mls)	0.0 (¯5 - 10)	0.0 (¯4.75 – 3.75)	0.3

24hr sp. >50%↑	19/38	12/52	0.01
24hr sp. >100%↑	15/38	10/52	0.05
Spont. sputum vol. (mls)	2.0 (0 – 4.5)	1.0 (¯1 - 3)	0.04*
Spont sp. >50% ↑	22/38	27/52	0.6
Spont sp. >100% ↑	21/38	26/52	0.67
Sputum colour (Units)	1.0 (0 - 1)	1.0 (0 - 1)	0.3
ISWT (m)	-60m (¯105 - ¯10)	-30 (¯80 - 5)	0.08
ISWT ≥ 5% ↓	28/38	27/52	0.049*

Table to show the median (IQR) difference in clinical parameters or number of patients with specified change in parameters based on bacterial load (BL) rise in 38 patients with ≥1 Log unit rise in bacterial load (BL) (CFU/ml) and 52 patients with <1 Log rise in CFU/ml from baseline to start of exacerbation. FEV_1:forced expiratory volume in 1 second, FVC: forced vital capacity, LCQ: Leicester cough questionnaire, SGRQ: St George's Respiratory Questionnaire, ISWT: Incremental shuttle walk test.

There were similar reductions in FEV_1 between the two groups. 60mls in those with higher bacterial load rise and 100mls in the less than 1 log CFU rise group. However, there were significantly more patients with a 10% or more reduction in FEV_1 and %predicted FEV_1 in the group with 1 or more unit rise in bacterial load (p=0.04). There was no significant difference between the groups in term of change in quality of life scores. There was a significant increase in the volume of spontaneous sputum production on exacerbation in the higher bacterial load group (P=0.04) but this was not seen in the 24-hour collection of sputum production or change in sputum colour. There was an overall larger reduction in exercise tolerance in the group with 1 or more unit log rise in bacterial load (60m versus 30m) but this did not reach statistical significance. However, there was significant difference in the number of patients that had an overall 5% reduction or greater in their exercise

tolerance between the groups with more people with reduced exercise tolerance by 5% or more in the 1 or more log unit rise in bacterial load group (74% versus 52%), p=0.049.

The improvement in lung function following antibiotics was small in both groups (50mls vs 60mls). There were small and similar improvements in both the Leicester Cough Questionnaire and the St George's Respiratory Questionnaire between the two groups. There was no difference between the two groups in the change in sputum colour or volume expectorated from start to end of exacerbation (Table 22).

Table 22.

VS⇒VE	≥1 LOG ↑ BL	<1 LOG ↑ BL	P VALUE
FEV_1 (L)	0.05 (¯0.03 – 0.15)	0.06 (¯0.07 – 0.14)	0.6
FVC (L)	0.07 (¯0.01 – 0.23)	0.10 (¯0.01 – 0.28)	0.9
LCQ (Units)	2.0 (0 - 5)	2.0 (0 – 4)	0.8
SGRQ (Units)	-1.0 (¯6.5 – 5.5)	0.0 (¯9.75 - 6)	0.9
24Hr sputum vol. (mls)	0.0 (¯10 – 3.5)	0.0 (¯7.25 - 5)	0.3
Spont. sputum vol. (mls)	-1.0 (¯4 – 0.5)	-1.0 (¯3 - 1)	0.4
Sputum colour (Units)	-1.0 (¯1 - 0)	-1.0 (¯1 - 0)	0.4
ISWT (m)	70 (25 – 100)	30 (10 – 85)	0.056

Table to show the median (IQR) differences in clinical parameters from start (VS) to end of exacerbation (VE) based on bacterial load rise at start of exacerbation. FEV_1:forced expiratory volume in 1 second, FVC: forced vital capacity, LCQ: Leicester cough questionnaire, SGRQ: St George's Respiratory Questionnaire, ISWT: Incremental shuttle walk test.

4.5.2 Blood inflammatory markers VO to VS

Both groups reported a rise in serum inflammatory markers but there was a significant difference between the two groups when assessing the degree of rise in white cell count and neutrophil count. Those with a 1 or more log unit rise in WCC

were more likely to double the white cell count or greater (11% versus 0%) (p=0.02) and increase the neutrophil count by 50% or more (45% versus 23%) (p=0.04).

There were similar increases in erythrocyte sedimentation rate between the two groups (p=0.9). There was a median increase in the CRP by 6 units in the higher bacterial load group compared to 2 units in the lower bacterial load rise but these results were not significant P=0.6 – see table 23.

Table 23.

VO⇒VS	≥1 LOG ↑ BL	<1 LOG ↑ BL	P VALUE
WCC (x10^9/L)	5.05 (2.67 – 7.3)	3.7 (0.78 - 7)	0.08
>25%↑	23/38	20/52	**0.05**
>50%↑	12/38	9/52	0.1
>100%↑	4/38	0/52	0.02*
Neutrophil (x10^9/L)	1.475 (0.22 – 3.53)	0.78 (-0.46 – 2.19)	0.03*
>25%↑	21/38	18/52	**0.05**
>50%↑	17/38	12/52	0.04*
>100%↑	8/38	7/52	0.3
ESR (mm/hr)	3.0 (-2 - 17)	3.0 (0 - 8)	0.9
>25%↑	18/38	23/52	0.8
>50%↑	17/38	19/52	0.5
>100%↑	12/38	8/52	0.07
CRP (mg/L)	5.0 (-0.5 - 29)	2.0 (0 -11)	0.5
>25%↑	20/38	29/52	0.8
>50%↑	20/38	26/52	0.49
>100%↑	20/38	24/52	0.26

Table of median (IQR) differences or number of patients with specified change in blood markers, depending on the degree of bacterial load (BL) rise (CFU/ml) from baseline (VO) to start of exacerbation (VS). WCC: White cell count, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein. *p<0.05.

There were similar improvements in the blood inflammatory markers after 14 days of antibiotics. The C-reactive protein results improved significantly more in the higher bacterial load rise group P=0.04. See table 24 for full results.

Table 24.

VS⇒VE	≥1 LOG ↑ BL	<1 LOG ↑ BL	P VALUE
WCC ($x10^9$/L)	-1.0 (¯3.2 – 0.1)	-0.60 (¯2.32 – 0.73)	0.3
Neutrophil ($x10^9$/L)	-0.79 (¯2.53 –0.24)	-0.36 (¯2.13 – 0.86)	0.2
ESR (mm/hr)	-1.0 (¯15 - 2)	-2.0 (¯10 - 2)	0.9
CRP (mg/L)	-8 (¯49 - 0)	0.0 (¯11 - 0)	0.04*

Table to show the median difference (IQR) in blood markers from start to end of exacerbation, depending on the degree of bacterial load rise at start of exacerbation. WCC: White cell count, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein. *p<0.05.

4.5.3 Sputum inflammatory markers

4.5.3.1 Myeloperoxidase (MPO)

There was a median (25^{th}, 75^{th} percentile) increase in MPO levels of 601μg/ml (238, 2834μg/ml) from baseline to start of exacerbation in the subset of patients whose bacterial load rose ≥1Log CFU. In the group of patients whose bacterial load changed <1Log CFU the median change was an overall decrease in myeloperoxidase by 61μg/ml CFU (-2695, 3166μg/ml) (P=0.04).

The median change of MPO of the two groups - ≥1Log rise in CFU and <1Log rise CFU after 14days of antibiotics (VS to VE) was an overall decrease of MPO by 1248μg/ml (-3743, 93.5μg/ml) and 712μg/ml (-5852, 0.0μg/ml) respectively. There was no difference between the two groups (P=0.7). The median (IQR) values at VO, VS and VE for each of the two subgroups are reported in table 25.

4.5.3.2 Neutrophil elastase (NE)

The change in neutrophil elastase from baseline to start of exacerbation significantly differed between the two groups. Those with a ≥1Log rise in bacterial CFU had a median increase of 437μg/ml (-62.5, 11250μg/ml) compared with 0.0μg/ml (-4489, 1394μg/ml) P=0.04 in the group with a less than 1 log increase in bacterial load.

There was a median decrease in neutrophil elastase levels after 14 days of antibiotics at the end of exacerbation visit (VS to VE) of 584μg/ml (-11136, 344μg/ml)) in the group with a higher bacterial load rise (≥1Log CFU) and decrease of 1100μg/ml (-3750, 0.0μg/ml) in the group with <1Log bacterial load rise, P=0.8. The median (IQR) values at VO, VS and VE for each of the two subgroups are reported in table 25.

4.5.3.3 Interleukin – 8 (IL-8)

There was no significant change in IL-8 levels from baseline to start of exacerbation (see figure 26 and table 20). In the subgroup analysis, there was no difference in IL-8 change between the higher and lower change in bacterial load groups (P=0.5). The median change was an overall decrease of 2833μg/ml (-17801, 24827μg/ml) in the ≥1Log CFU and decrease of 5414μg/ml (-16234, 4373μg/ml) in the <1Log CFU group.

At the end of exacerbation both groups responded to 14 days of antibiotic treatment with a decrease in IL-8 levels. There was a median reduction of 2565mg/ml in the ≥1Log rise in CFU group and 1736mg/ml in the <1Log rise in CFU group, P=0.5. The median (IQR) values at VO, VS and VE for each of the two subgroups are reported in table 25.

Table 25.

Marker	≥1log rise			<1log rise		
	VO	VS	VE	VO	VS	VE
25th percentile MPO	0.0	745.8	315.0	785.0	692.0	0.0
Median MPO (μg/ml)	990.5	2803	798.0	1609	2307	593.0
75th percentile MPO	2846	11427	4582	6545	6938	1651
P value VO⇒VS	0.0006***			0.8		
P value VS⇒VE		0.018*			0.0004***	
P value VO⇒VE	0.86			<0.0001***		
25th percentile NE	338.0	2284	1692	1879	2044	0.0
Median NE (μg/ml)	2725	3350	2538	2850	3819	2000
75th percentile NE	5267	32116	4871	6869	5485	2850
P value VO⇒VS	0.028*			0.97		
P value VS⇒VE		0.06			0.0005***	
P value VO⇒VE	0.9			0.0006***		
25th percentile IL-8	4048	5358	4285	5049	5322	0.0
Median IL-8 (pg/ml)	12306	16816	9813	18921	13883	5685
75th percentile IL-8	34025	28334	25746	31200	21598	22175
P value VO⇒VS	0.99			0.09		
P value VS⇒VE		0.095			0.35	
P value VO⇒VE	0.1			0.08		

Table to show the median, 25th and 75th percentile values for sputum inflammatory markers MPO: myeloperoxidase, NE: neutrophil elastase, IL-8: interleukin-8. P values calculated using the Wilcoxon t-test for the subgroups ≥1log rise and <1log rise in bacterial load at start of exacerbation. VO: baseline, VS: start of exacerbation, VE: end of exacerbation. *P<0.05, ***p<0.001.

4.5.4 Symptoms

At the start of exacerbation only, patients were asked to report any new symptoms they experienced. Most patients (91%) reported increased or new cough. There were changes in sputum characteristics on exacerbation with 78% of patients reporting increased sputum volume and 72% of patients reporting increased sputum purulence. 79% of patients reported feeling more breathless than usual. See table 26 for full results. Symptoms were also analysed to see if there was a difference between patients with a ≥1 log rise in bacterial load CFU and those that had a less than 1 log rise. Both groups had a similar distribution of symptoms except those in the higher bacterial load category were more likely to develop pleuritic chest pain ($p=0.04$) – table 26.

Table 26.

	N=92 Total exacerbations n (%)	N=37 ≧1 Log BL n (%)	N=52 <1 Log BL n (%)	P value
Worsening Cough	**84 (91)**	**35 (95)**	**45 (87)**	**0.3**
Increased sputum volume	**72 (78)**	**31 (84)**	**35 (67)**	**0.09**
Increased sputum purulence	**69 (75)**	**31 (84)**	**34 (65)**	**0.08**
Breathlessness	**73 (79)**	**30 (81)**	**39 (75)**	**0.6**
Fatigue	**71 (77)**	**29 (78)**	**37 (71)**	**0.5**
Fever	**40 (43)**	**17 (46)**	**19 (37)**	**0.4**
Chest pain	**36 (39)**	**19 (51)**	**15 (29)**	**0.046***
Wheeze	**48 (52)**	**20 (54)**	**23 (44)**	**0.4**
Stridor	**2 (2)**	**0 (0)**	**1 (2)**	**1.0**
Haemoptysis	**9 (10)**	**2 (5)**	**7 (13)**	**0.3**
Sore throat	**35 (38)**	**14 (38)**	**19 (37)**	**1.0**
Coryzal symptoms – nose/ears	**50 (54)**	**19 (51)**	**27 (51)**	**1.0**
Headaches	**41 (45)**	**13 (35)**	**25 (52)**	**0.3**
Myalgia	**39 (42)**	**16 (43)**	**21 (40)**	**0.8**
Rigors	**34 (37)**	**14 (38)**	**20 (38)**	**1.0**
Diarrhoea&Vomiting	**11 (12)**	**4 (11)**	**5 (10)**	**1.0**

Table to show the different symptoms patients experienced on start of exacerbation. 2 patients did not complete symptom questionnaires. 3 patients did not have either a start or an end sputum load calculated. Results presented as N (%). Chi squared analysis to see if patients with 1 or more log unit rise in bacterial load were more likely to present with each symptom.

Taking the univariate results into account, clinically important symptoms and those with a p value less than 0.3 were included in a multivariate backward logistic regression to see if they were predictive of a 1 or more log unit rise in bacterial load. Cough was not included because sputum volume and purulence (surrogate markers of cough) were included. Results shown in table 27 demonstrate chest pain, increase in sputum volume and the absence of headache are more likely to be associated with an increase in bacterial load at start of exacerbation.

Table 27.

SYMPTOM	ODDS RATIO (OR)	95% CI	P value
Chest pain	**3.95**	**1.42-10.97**	**0.008***
Haemoptysis	**0.22**	**0.04-1.31**	**0.096**
Headache	**0.33**	**0.12-0.92**	**0.034***
Increase in sputum purulence	**2.76**	**0.90-8.49**	**0.08**
Increase in sputum volume	**3.18**	**1.01-10.07**	**0.049***

Table to show the odds ratio with 95% confidence intervals (CI) of each symptom in the multivariate backward logistic regression model. The dependent variable was a 1 log unit rise in bacterial load.

Guidelines in COPD have recommended antibiotics for exacerbations based on the simple Anthonisen criteria of 3 patient reported symptoms of increased sputum volume, increased sputum purulence and increased dyspnoea. Similarly, the British Thoracic Society (BTS) guidelines have a similar definition of an exacerbation including increased sputum purulence, increased sputum volume or viscosity and increased cough and/or wheeze and/or dyspnoea and/or systemic upset (figure 5). Symptom complexes were assessed to see if they are more likely to affect bacterial load rise with exacerbations - table 28. There was a statistically significant

likelihood of having a 1 or more log unit rise in bacterial load CFU/ml if patients developed the triad of symptoms (cough, increased sputum volume, increased sputum purulence) at start of exacerbation (p=0.005). There was also a significant likelihood of having a 1 or more unit rise in bacterial load if the patients had the above triad of symptoms as well as chest pain (p=0.0004) – see table 28. Taking the multivariate results into account, the symptom complex of chest pain, increased sputum volume without headache was also significantly more likely to predict a rise in bacterial load. There was no significant difference, when assessed by chi square, in the likelihood of having symptom complex of 'increased cough, sputum volume and purulence' than having 'chest pain, increased sputum volume without headache' with a 1 or more log unit rise in bacterial load, p=0.17.

Table 28.

SYMPTOMS	N=92 Total exacerbations n (%)	N=38 ≧1 Log BL n (%)	N=52 <1 Log BL n (%)	P value
↑Cough + ↑sp. Vol + ↑sp. Purulence	**51 (55)**	**27 (73)**	**22 (42)**	**0.005**
↑Cough + ↑sp. Vol + ↑sp. Purulence + chest pain	**20 (22)**	**15 (41)**	**4 (8)**	**0.0004**
↑sp. Vol + chest pain - headache	**11 (12)**	**9 (24)**	**2 (4)**	**0.0073**
↑Purulent sp. AND ↑Cough OR ↑sp. Vol	**65 (71)**	**29 (78)**	**32 (62)**	**0.1**
↑Purulent sp. + chest pain	**26 (28)**	**16 (43)**	**9 (17)**	**0.009**
↑sp.Vol + chest pain	**28 (30)**	**18 (47)**	**9 (17)**	**0.0027**

Table to show if symptom complexes were more likely to be associated with a 1 or more log unit rise in bacterial load CFU/ml. Analysis performed by chi-squared. Sp: sputum.

The different definitions of an exacerbation along with the outcome from the above multivariate model were used to assess the sensitivity, specificity, positive and negative predictive values of a 1 or more log unit rise in bacterial load for each symptom complex. The model from the multivariate analysis results need to be externally validated before comparison with accepted definitions by either the British Thoracic Society or European Respiratory Society can be made to see if it has improved specificity. Sensitivity, specificity, positive and negative predictive values from this cohort for different symptom complexes are shown in Table 29.

Table 29.

SYMPTOM COMPLEX	Sensitivity	Specificity	Positive predictive value	Negative predictive value
British Thoracic Society definition	**71%**	**58%**	**55%**	**73%**
European Respiratory Society consensus definition	**95%**	**10%**	**43%**	**71%**
↑sp. Vol + chest pain - headache	**24%**	**96%**	**82%**	**63%**
↑Purulent sp. AND ↑Cough OR ↑sp. Vol	**76%**	**38%**	**48%**	**69%**
↑Purulent sp. + chest pain	**42%**	**83%**	**64%**	**66%**
↑sp. Vol + chest pain	**47%**	**83%**	**66%**	**68%**

Table to show the sensitivity, specificity, positive and negative predictive values for each symptom complex. British thoracic society definition: worsening of cough, increased sputum volume or viscosity, increased sputum purulence with or without increasing wheeze, breathlessness, haemoptysis. The European consensus: deterioration in three of more of: cough; sputum volume or consistency; sputum purulence; breathlessness and/or exercise tolerance; fatigue and/or malaise; haemoptysis. Sp: sputum.

4.5.5 Virology

Many patients reported symptoms that could be attributable to viral infection - 54% of patients reported developing coryzal symptoms of runny or blocked nose and blocked ears, 37% developed a sore throat, 43% of patients reported myalgia, 44% reported headache and 11% reported new diarrhoea and/or vomiting (table 26).

Patients that developed an exacerbation had a viral throat swab sent for routine virology. 90 viral throat swab tests were performed and of these, 16 (18%) were reported as positive for viral infection. The viruses detected are outlined in table 30.

Table 30.

Virus	No. of positive results
Rhinovirus	12
Influenza A	2
Influenza B	1
Parainfluenza	1

Table of positive viral results from 16 of total 90 exacerbations tested.

Results were analysed to see if positive viral infection affected the bacterial load cultured on sputum microbiology. There was no significant difference in the number of viral positive samples between the higher ≥1 log bacterial CFU rise and < 1Log rise in bacterial CFU groups, p=0.6 – see table 31.

Table 31.

	Positive for viral infection	Negative for viral infection
≥1 log rise in bacterial load CFU	5	32
<1 log rise in bacterial load CFU	10	42

Table to show that the detection of viruses had no impact on bacterial load, Chi squared analysis, p=0.6.

4.6 Discussion

Exacerbations are currently subjectively defined based on symptoms. The British Thoracic Society (BTS) Guidelines defined an exacerbation as an acute deterioration (usually over several days) with worsening local symptoms (cough, increase sputum volume or change of viscosity, increased sputum purulence with or without increasing wheeze, breathlessness, haemoptysis) and/or systemic upset (Pasteur *et al*, 2010). The recent European consensus led by Hill and colleagues concluded an exacerbation to be when a patient exhibits a deterioration in three of more of the following key symptoms for at least 48hours: cough; sputum volume and/or consistency; sputum purulence; breathlessness and/or exercise tolerance; fatigue and/or malaise; haemoptysis AND a clinician determines that a change in bronchiectasis treatment is required (Hill *et al*, 2017). Treatment in the form of antibiotics is given based on BTS definition in the outpatient setting with no immediate access to blood test results to check inflammatory markers or chest x-rays to investigate for consolidation.

This study investigated exacerbations of bronchiectasis that could be managed on an outpatient basis to see if the exacerbations could be phenotyped and the role of quantitative sputum bacteriology and its implications explored further. 94 Patients were recruited whilst clinically stable. These patients performed baseline tests to measure pulmonary function, exercise tolerance, quality of life and blood and sputum inflammatory markers. Patients were asked to return when they felt they were experiencing an exacerbation to repeat the tests and receive a prescription for 14days of oral antibiotics based on previously sputum microbiology. Patients returned after 2 weeks to repeat all investigations again.

Based on the subjective definition of exacerbation, patients that thought they were experiencing an exacerbation had a significant reduction in lung function tests, increase in blood inflammatory markers, reduced exercise tolerance and a reduction in quality of life as assessed by the Leicester Cough Questionnaire and the St George's Respiratory Questionnaire at start of exacerbation when compared with

baseline results. Sputum myeloperoxidase and free neutrophil elastase were also significantly elevated but interleukin-8 did not significantly change.

The sputum microbiology showed that more patients cultured a pathogen in their sputum at the start of exacerbation compared to baseline and they were also more likely to culture multiple pathogens. There was an increase in the number of positive culture results for *Pseudomonas aeruginosa*, other gram negative pathogens and other potentially pathogenic microorganisms with a reduction in culture negative results or those that cultured mixed normal flora at start of exacerbation. There were significantly more culture results that grew a different dominant pathogen from that which was cultured at baseline for the same patient. A dominant pathogen was defined as the pathogen with the highest bacterial load if more than 1 microorganism was identified.

Quantitative sputum bacteriology also showed that there was a significant around 2 log increase in the bacterial load from a mean baseline of 10^5 colony forming units (CFU) to 10^7 CFU at start of exacerbation. If the dominant pathogen cultured were to change at visit start then this was more likely to be associated with a 1 or more log rise in bacterial load ($p=0.02$). If the dominant pathogen did not change then there was no significant rise in bacterial load from baseline to start of exacerbation ($p=0.9$). A small number of patients ($n=12$) tested positive for a virus at start of exacerbation, the majority of these were diagnosed as rhinovirus, but the latter did not impact on bacterial load rise.

Lastly, the implications of a 1 or more unit log rise in bacterial load in a clinical setting was explored. A bacterial load rise of 1 or more log CFU was associated with a deterioration of 10% in actual and predicted FEV_1. It was also significantly associated with an increase in volume of spontaneous sputum expectorated. Blood inflammatory markers were also investigated and an increase in bacterial load of 1 log unit or more was significantly associated with a 100% rise in white cell count and a 50% increase in neutrophil count. It was also associated with an increase in sputum inflammatory markers myeloperoxidase and neutrophil elastase when

compared with those with a less than 1 log unit rise in CFU. Out of all those that had a 1 or more log unit rise in bacterial load 79% had a change in dominant pathogen (p=0.0006). The presence of viruses did not have an impact on bacterial load.

Patients presented with various symptoms as outlined in table 24. A univariate analysis showed 'chest pain' to be significantly associated with a 1 or more unit rise in bacterial load on exacerbation. A multivariate backward logistic regression analysis showed chest pain as well as an increase in sputum volume and the absence of headache to be associated with a 1 or more log unit rise in bacterial load CFU/ml. Patients presenting with a combination of symptoms can also help differentiate a rise in bacterial load. The results from the multivariate model identified increase in sputum volume, the presence of chest pain and the absence of headache to be much more specific for a rise in bacterial load count than either of the BTS or ERS consensus definitions for exacerbation. The addition of chest pain to either increased sputum purulence or to increased sputum volume increased the specificity for recognising a rise in bacterial load of 1 or more log units. 'Chest pain' is a symptom absent from both the British thoracic society and ERS consensus definitions.

National definitions such as those described above by the BTS and ERS consensus definition do not discriminate between inpatient and outpatient exacerbations and are largely based on grade D expert opinion alone. There is a paucity of studies investigating outpatient exacerbations of bronchiectasis. Symptoms associated with a rise in bacterial load in this outpatient cohort are chest pain, increase in 24hr sputum volume and the absence of headache. This symptom complex has a high specificity but lower sensitivity. Therefore, it may be more productive to use this to help exclude disease rather than to diagnose it as a higher proportion of patients that did not have this triad of symptoms did not have a rise in bacterial count above 1 log unit.

This study showed exacerbations to be associated with a rise in bacterial load but this conflicts with the study by Tunney and colleagues (2013) who investigated bacterial load and bacterial community present in a small number of patients (n=12)

with an exacerbation who were treated with intravenous antibiotics. They found bacterial loads at start of exacerbation to be similar to when clinically stable and that there was only a limited shift in microbial taxa. This was a small study in a more severe cohort of patients and could reflect the difference. Cox *et al* also investigated bacterial load and found no change in bacterial communities or change in bacterial load from baseline, start and end of exacerbation. This cohort of patients were more severe than the patients in this thesis with a higher percentage of patients culturing Pseudomonas (45%). They, therefore are more likely to have had more exacerbations and antibiotics in the past which could account for the change in resident bacteria. The study was also small examining only 13 paired baseline and exacerbation samples (Cox et al, 2017). Results from this outpatient cohort reveals the rise in bacterial load to be attributable to a change in primary dominant pathogen and this could be a result of them being a relatively antibiotic naive population. There was no change in bacterial load at start of exacerbation if the dominant pathogen remained the same as that which was cultured at the baseline visit when patients were clinically stable. There is evidence that would support the change in bacterial communities as a result of certain bacteria e.g. *Pseudomonas* and *Haemophilus* (Cox *et al*, 2017). To the best of our knowledge this is the first study to demonstrate the importance of a change in dominant pathogen and could have implications for the way we investigate and treat exacerbations.

The hypothesis that a 1 or more log unit rise in bacterial load would be associated with a more severe exacerbation was investigated. This study highlighted that there is increased sputum volume and purulence as well as poor quality of life in all patients that experienced an exacerbation but there did not appear to be an association with increased bacterial load rise of 1 or more log unit in CFU/ml. However, there was a significant association with a rise in bacterial load of 1 or more log units and reduction in distance walked by 5 or more percent in the incremental shuttle walk test - a test that objectively measures functional capacity. It was also associated with a 10% or more reduction in both lung function parameters actual FEV_1 and %predicted FEV_1 as well as volume of spontaneous sputum expectorated. As expected both groups in the subanalysis (1 or more log unit rise

and less than 1 log unit rise in bacterial load) improved with antibiotic therapy and there was little to differentiate the improvement apart from a greater reduction in c-reactive protein in the higher bacterial load group (p=0.049).

4.7 Limitations

There were several limitations to this study. This study was based on outpatient exacerbations and therefore were less severe and did not require hospital admission. The applicability of these results and conclusions will require further investigation for inpatients and severe exacerbations. Patients were asked to telephone when they experienced an exacerbation during normal working hours, thus some exacerbations will have been missed.

Standardised qualitative and quantitative microbiology techniques were used but not molecular microbiology techniques such as 16S rDNA pyrosequencing. A study by Tunney *et al* and Cox *et al* suggested there was poor overall agreement between standard microbiology and 16S rDNA pyrosequencing. They found 150,000 sequences from 40 sputum samples and reported the majority of them were from taxa present in low abundance and that the community present did not change from stable, to start of exacerbation or indeed after treatment with antibiotics.

4.8 Significance

This study investigating outpatient exacerbations may have implications for the way we investigate and treat outpatient exacerbations. If higher bacterial loads lead to more severe exacerbations, then there could be an argument for assessing the need for intravenous antibiotics based on bacterial load. Antibiotic choice is currently prescribed based on previous microbiology data but if the dominant pathogen changes at start of exacerbation then how we treat exacerbations may require further investigation.

4.9 Conclusion

In this cohort of outpatient exacerbations of bronchiectasis there was a 2-log increase in bacterial load as well as a deterioration in lung function, exercise

tolerance, quality of life and an increase in sputum and serum inflammatory markers at start of exacerbation. Those with a 1 or more log unit rise in bacterial load count at start of exacerbation can exhibit more severe characteristics, present with symptom complexes including chest pain but not headache and are likely to have a change in the dominant pathogen. Exacerbations do lead to a rise in bacterial load and that this is due to a change in dominant pathogen.

Chapter 5:
Validating the incremental shuttle walk test

5.1 – The Incremental Shuttle Walk Test (ISWT)

Bronchiectasis is a chronic lung condition where patients commonly experience exacerbations and progression of disease. The mainstay of treatment consists of antibiotics and chest physiotherapy but novel therapies are being assessed to break the vicious circle of bronchiectasis.

There is an urgent need for objective endpoints to assess progress and response to treatment in bronchiectasis. 24-hour sputum volume, microbial clearance, C-reactive protein, quality of life assessed by the SGRQ (Wilson *et al,* 1997b) and the LCQ (Murray *et al,* 2009d) have been found to be useful clinical endpoints (Murray *et al,* 2009c). New therapies are continually being investigated in bronchiectasis to break the vicious circle of disease and need evaluation. 'Exacerbation frequency' or 'time to next exacerbation' is now the endpoint of choice used in phase 3 randomised controlled trials (Haworth *et al* 2014, Fan *et al,* 2015, Wong *et al,* 2012, Serisier *et al,* 2013) but there was uncertainty on the definition of what an exacerbation is. Recently, a European Consensus statement was published to try define what an exacerbation is for use in clinical research (Hill *et al*, 2017).

24-hour sputum volume is thought to be inaccurate and unreliable. It is onerous and often embarrassing for the patient to collect. In clinical practice this test is poorly complied with and therefore difficult to interpret. In addition, it takes, by definition 24 hours to assess response.

Microbial clearance has been used to assess response to antibiotic treatment but often in clinical practice only qualitative analysis has been performed. Quantitative microbiological analysis has currently only been carried out in phase 2 trials (Serisier *et al* 2013b). The lungs, once thought to be sterile, are now theorised to contain lung microbiota and the presence of microorganisms cultured could represent colonisation or infection. Some facilities offer quantitative microbiological analysis but a change in bacterial load is not universally thought to increase with exacerbations or decrease with treatment (Tunney *et al, 2013 &* Cox *et al, 2017*).

A subset of patients routinely culture mixed normal flora or respiratory commensals on sputum qualitative culture at the start of exacerbation. Microbial clearance would not be a suitable endpoint for these patients or in those that cannot expectorate. In addition, microbial culture takes at least 24 hours to perform so results are not readily available at the bedside.

C-reactive protein (CRP) can be useful in patients with a significant systemic response to infection but the degree of inflammatory response in patients cannot be predicted and is often varied. It is less useful in those without a systemic response to infection and is of limited value in patients that are clinically stable. As such, it has not been used to assess longitudinal decline in bronchiectasis or any long-term therapies for bronchiectasis. CRP is a relatively non-specific marker of inflammation which can often lag behind the clinical picture.

Several quality of life questionnaires have been validated for use in bronchiectasis; SGRQ, (Wilson *et al,* 1997b) LCQ (Murray *et al*, 2009d), Bronchiectasis health questionnaire (Spinou *et al,* 2017) & the Quality of life questionnaire-bronchiectasis (QOL-B) (Quittner *et al*, 2014 & Quittner *et al,* 2015). These are more subjective outcome measures incorporating patient self-assessment and reporting. The questionnaires can be cumbersome for the patient to complete and time-consuming for the clinician to analyse.

A validated endpoint that is reliable, quick and easy to perform would help evaluate existing and new therapies. The incremental shuttle walk test is an assessment of functional exercise capacity and the previous two chapters in this thesis have shown that ISWT correlates well with disease severity and deteriorates with exacerbation and improves after treatment. Taking these interesting findings into account, coupled with the fact there is currently no validated marker of functional capacity in bronchiectasis, the aim of this chapter is to assess if the ISWT could be a reliable indicator of longitudinal decline in bronchiectasis and whether it could be used to measure response to treatment by validating it for use in bronchiectasis.

Improving exercise tolerance and preventing deconditioning is an important therapeutic goal in chronic respiratory disease (Palange *et al*, 2000). Therefore, as part of the long-term outpatient management for COPD, patients are routinely referred for pulmonary rehabilitation to improve exercise tolerance and capacity. The incremental shuttle walk test has been tested in chronic obstructive lung disease (COPD) (Singh *et al,* 1992), cystic fibrosis (Bradley *et al,* 2000), interstitial pneumonias (Eaton *et al*, 2005) and advanced cancers (Booth *et al*, 2001). A study performed in 372 patients with COPD were asked to perform the incremental shuttle walk test (ISWT) before and after completing a 7 week pulmonary rehabilitation course. They found the minimum clinically important difference (MCID) to be 47.4m with patients also recognising an additional benefit in their COPD state when able to walk a further 78.7m (Singh *et al*, 2008).

It has also been used to assess the effect of interventions on exercise capacity and response to new therapies such as antibiotics (Murray *et al,* 2011), chest physiotherapy (Murray *et al,* 2009b) and pulmonary rehabilitation (Mandal *et al,* 2012) in bronchiectasis. A randomised crossover trial of 20 patients with bronchiectasis underwent 3 months of chest physiotherapy followed by a 3 month period of no physiotherapy after a 1 month washout period. They found a statistically significant improvement in the distance walked in the ISWT by 40m in the treatment group (p=0.001) (Murray *et al*, 2009b). Patients with bronchiectasis can also present with fatigue and breathlessness and improving exercise capacity might help alleviate these symptoms in this chronic respiratory disease. Another study in patients with bronchiectasis and limited exercise tolerance showed an increase in ISWT by 56.7m (p=0.03) after 8 weeks of pulmonary rehabilitation and the improvement was sustained 12 weeks post the end of pulmonary rehabilitation (80m) when compared with controls (p=0.04) (Mandal *et al*, 2012). The incremental shuttle walk test was also used to assess the impact of inspiratory muscle training in addition to pulmonary rehabilitation. 32 patients were randomised to either 1) pulmonary rehabilitation with inspiratory muscle training, 2) pulmonary rehabilitation with sham inspiratory muscle training or 3) control group for eight weeks. The authors found pulmonary rehabilitation improved the ISWT

significantly by 124.5m with inspiratory muscle training and 96.7m without. The was no significant difference between the groups (p=0.22) (Newall *et al*, 2005).

A small study conducted in patients with bronchiectasis investigated the MCID in this patient group. 37 patients were asked to perform the ISWT pre- and post- an eight week exercise programme. They found the MCID to be 35m (AUC 0.88, 95% CI 0.73-0.99)) using the Anchor based method and 37m using the distribution method. They concluded that small changes in the ISWT score were likely to represent clinically important benefits (Lee *et al*, 2014c).

The above studies show that the incremental shuttle walk test has been used in bronchiectasis but it has never to date been fully validated for use. The incremental shuttle walk test is a relatively inexpensive test, easy to perform and quick to analyse. A validated endpoint to objectively assess a patient's functional status would be helpful to see if patients have responded to treatment and returned to their baseline physical capability, and whether like COPD patients, they could benefit from pulmonary rehabilitation or other forms of exercise in the long-term management of chronic disease.

5.2 – The validation process

5.2.1 Incremental shuttle walk test

The ISWT was performed according to standard test procedure (Singh *et al,* 1992). The patient is asked to walk a 10-metre shuttle course on a flat surface with the walking speed controlled using pre-recorded audio signals. There are twelve incremental levels with each level lasting one minute. The pre-recorded audio signals beep progressively faster so that more distance is covered at each level. The test continues until such a time that the participant is unable to complete the shuttle in the designated time (0.5metres or further from the marker) at the time of the audio signal) or the patient becomes too breathless to continue. The distance completed is recorded. All patients were instructed to walk at a steady pace walk aiming to turn around at each end of the course at the sound of the audio signal. All were advised to continue to walk until they felt unable to maintain the required speed without

becoming unduly breathless. At the start and end of the ISWT all patients had their heart rate, oxygen saturations and dyspnoea score according to the modified BORG dyspnoea scale (Borg, 1982) in accordance with standardised guidelines (2002).

The MCID has been proposed as 35m but successful completion of distance walked in this study was recorded in increments of 10m. Difference in ISWT walked between visits are presented in groups of 'deterioration/no change', '1-29m', '30-59m', '60-89m' and '≥90m'. As baseline distance varied amongst patients, the percentage change in ISWT distance was also calculated and presented in groups of 'deterioration', 'no change', '0-4.99%', '5-9.9%' and '≥10%' change.

5.2.2 Study design

In order to validate the incremental shuttle walk test as a useful endpoint in bronchiectasis, the reliability, validity and responsiveness needs to be demonstrated. The minimum clinically important difference (MCID) was also investigated.

Each section was assessed separately by using several studies as outlined below. Every patient in each of the studies had previously performed the ISWT as part of routine clinical practice and was familiar with how the test was conducted and so a practice test was not necessary.

The reliability of the ISWT was assessed by inviting 30 sequential patients, a number thought to be acceptable to the researchers with bronchiectasis (varying severity), to complete the incremental shuttle walk test 6 months apart when they were clinically stable. There was no change made to their medical management of bronchiectasis during this time.

The validity of the ISWT was assessed by correlating the distance walked by patients with the SGRQ scores – a validated marker of quality of life that assess activity as one of its scoring domains. ISWT results were correlated with SGRQ total scores and SGRQ activity scores in 94 patients at three different time points: when clinically stable, at start of an exacerbation and at the end of an exacerbation

after 14 days of oral antibiotics treatment, prescribed according to previous microbiology results.

In a substudy, 49 patients of the above cohort were asked to wear an activity monitor continuously for a period of 8 days, only removing to bath or shower. Data from the physical activity monitors were correlated with the ISWT to assess functional capacity. ISWT results were also correlated with MRC breathlessness scores (Bestall *et al*, 1999) and BSI scores (Chalmers *et al*, 2014) to assess disease severity.

To assess responsiveness, the ISWT was performed in patients that were treated with either short-term (14 days) intravenous or oral antibiotic therapy for an exacerbation of bronchiectasis. A repeat analysis from a previous study assessing the impact of long-term nebulised gentamicin twice a day for 12months compared with placebo (0.9% saline) on the ISWT was performed (Murray *et al*, 2011).

30 patients that required intravenous antibiotic therapy for an acute exacerbation of bronchiectasis were asked to perform the test pre- and post- 14 days of antibiotic therapy. These patients had to meet the criteria for receiving intravenous antibiotics which included having failed oral treatment, a severe exacerbation necessitating inpatient stay or sputum microbiology results necessitating an intravenous agent to be administered (Pasteur *et al*, 2010).

94 patients that required oral antibiotic therapy for an acute exacerbation of bronchiectasis that could be managed on an outpatient basis were asked to perform the test pre (Visit start, VS) and post 14 days of antibiotic therapy (Visit end, VE). Antibiotic choice was guided by previously microbiology results. These patients also performed the ISWT when clinically stable at least 30 days pre-exacerbation (Baseline visit, V0).

In the long-term nebulised antibiotic group, 30 patients with bronchiectasis who were treated with nebulised gentamicin 80mg, b.i.d. for 12 months performed the

ISWT prior to starting treatment and at 12months. 30 patients that were treated with 12 months of nebulised 0.9% saline performed the ISWT similarly (Murray *et al*, 2011).

5.2.3 The minimum clinically important difference

The anchor method was used to determine the minimum clinically important difference using a 4 or more unit rise in SGRQ as a clinically significant improvement of quality of life (Wilson *et al*, 1997b). The 1-year gentamicin study (Murray *et al*, 2011) was reanalysed to assess the area under the curve (percent change of ISWT with a 4 or more-unit improvement in total SGRQ). This study was selected as there was significant improvement of 1-year nebulised gentamicin compared with the group that received nebulised saline.

5.2.4 Lung function

Pre-bronchodilator Forced expiratory volume in 1 second (FEV_1), Forced vital capacity (FVC) and ratio FEV_1:FVC by spirometry as per standardised protocols (Miller *et al*, 2005). Patients were asked to take three attempts and the best results were recorded. All patients have previously performed spirometry as part of routine clinical practise.

5.2.5 Sputum

Sputum colour was recorded according to a visual colour chart rating. Mucoid sputum was rated 1, mucopurulent rated as 2 and purulent sputum rated as 3 or 4 depending on colour (Murray *et al*, 2009). 24hour sputum volume was recorded by asking patients to expectorate solely into a universal container for 24hours. Qualitative and quantitative microbiology was performed on all samples pre- and post 14 days of antibiotics.

5.2.6 Statistical analysis

All data were analysed using Graphpad prism version 5.0a (Graphpad software, San Diego, CA, USA). For demographic and clinical variables, data are presented as median (interquartile range) for continuous variables and n (%) for categorical

variables unless specified otherwise. Comparison of changes within a group i.e. before and after treatment was calculated using Wilcoxon signed rank test. We compared categorical data between groups with the $\chi 2$ test. A P value <0.05 was considered statistically significant for each analysis. A Bland-Altman test was used to show repeatability over time with bias and 95% limits of agreement calculated and intraclass correlation coefficient calculated. Correlations were assessed using the Spearman correlation. An unpaired *t* test was used to calculate the change from baseline to 12months in ISWT between patients assigned gentamicin and nebulised saline with results displayed as mean difference (95% confidence interval).

5.3 Results

5.3.1 Reliability

30 patients were asked to perform the incremental shuttle walk test when clinically stable and then repeat the test in 6 months' time. No changes were made to their medical management. The aetiology of their bronchiectasis varied from 70% idiopathic, 13% post infection, 13% autoimmune disease and 3% IgG2 subclass deficiency. Their baseline characteristics are outlined below in table 1.

Table 1.

Characteristics	
Age	59.1 (11.4)
Female	47%
Smoking	60% never 40% ex-smoker 0% current
FEV_1 Predicted FEV_1	2.2L (0.9) 73.9% (24.5)
FVC Predicted FVC	3.2L (1.1) 86.4% (25.6)
Sputum microbiology	40% potentially pathogenic microorganisms 57% mixed normal flora 3% no sputum
Inhaled/ oral corticosteroids	60% 7%
COPD	0%
Asthma	5.7%
Long-term antibiotics	3%

Results presented as mean (SD) or % of patients. FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, COPD: chronic obstructive pulmonary disease.

At the first visit, patients walked a median distance of 390m. 6 months later patients were recalled and the test was performed again in an identical manner. A median distance of 400m was walked. There was no significant difference in the distance

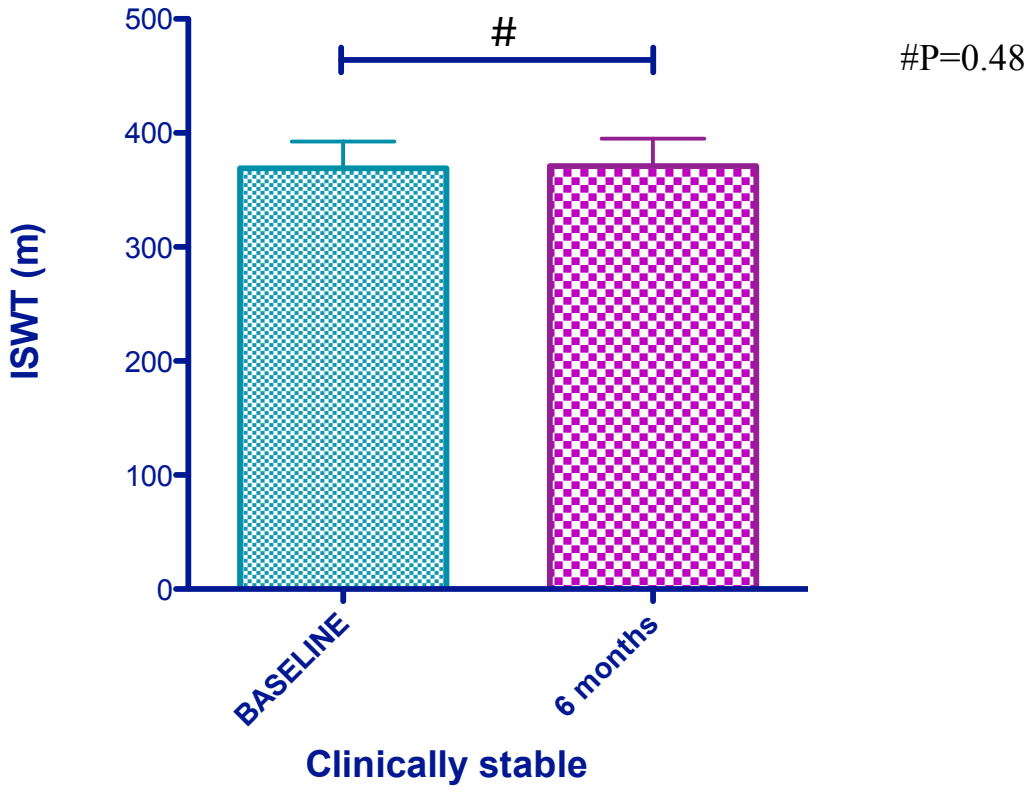
walked in these clinically stable patients (see table 2 and figure 1). The median change in distance walked was 0m (-25m – 50m) with median percentage change 0% (-4.9% - 18.2%).

Table 2.

Value	Baseline distance (m)	6-month distance (m)
25% Percentile	225.0	260.0
Median	390.0	400.0
75% Percentile	462.5	480.0

Table to show the median and interquartile ranges of distances walked in 30 clinically stable patients with bronchiectasis.

Figure 1.



Barchart of mean (SEM) to show there is no significant difference in distance (m) walked after 6 months in clinically stable patients with bronchiectasis. ISWT: incremental shuttle walk test, #p>0.05.

A Bland-Altman plot showed a bias of 1.38m and 95% limits of agreements from -188m to 185m (see figure 2). The intraclass correlation coefficient was calculated to be 0.85 (95% CI 0.68-0.93, p<0.001).

Figure 2

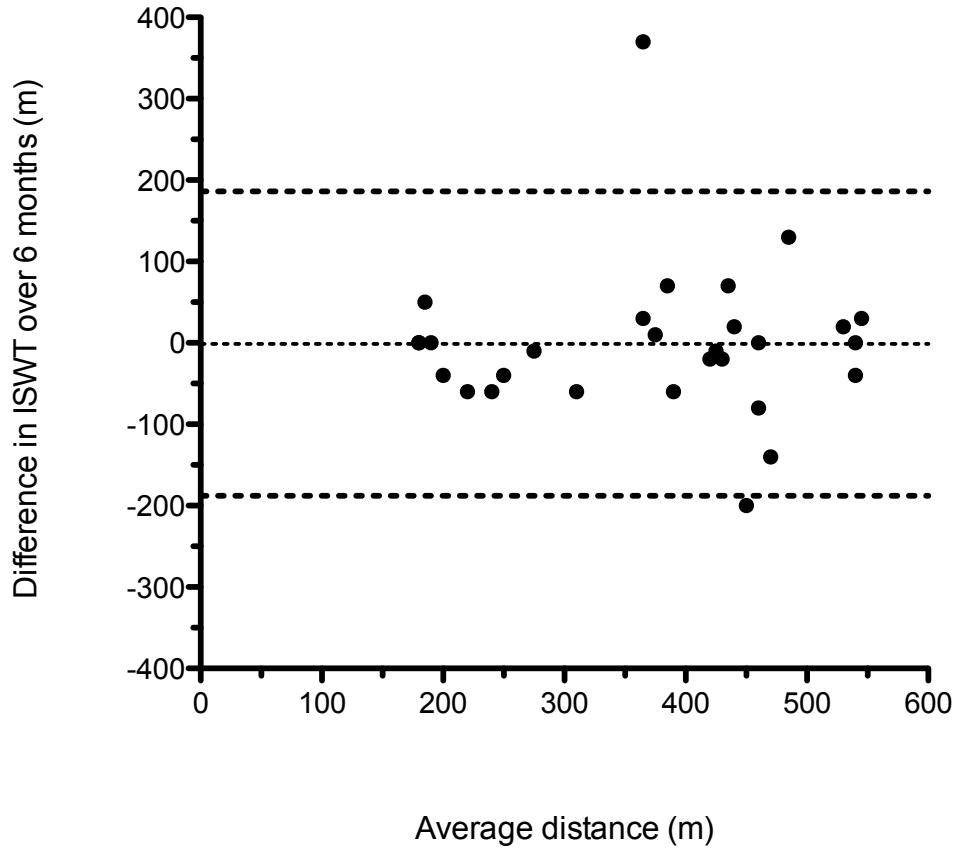


Figure 2. Bland-Altman figure of 30 stable patients that completed the ISWT 6 months apart. The difference versus average distances walked are shown. The middle line represents the mean bias (-1.4). The upper and lower dashed lines represent the limits of agreement (95% confidence intervals -188.4 – 185.6).

5.3.2 Validity

To assess the validity of the incremental shuttle walk test, it needs to be comparable with other already validated markers of function in bronchiectasis. To assess this, we correlated the results of 94 patients' incremental shuttle walk test results with

their St George's Respiratory Questionnaire (SGRQ) results which has already been validated for use in bronchiectasis (Wilson *et al*, 1997b). The SGRQ scores 'activity' as part of its evaluation and hence correlations between 'Total SGRQ' and 'Activity SGRQ' with the ISWT were assessed.

The SGRQ changed with exacerbations: the median values at baseline for SGRQ were 28 (18-44) to 36 (25-36) at visit start ($p<0.0001$). The median then reduced to 34 (20-51.75) at visit end ($p=0.003$) – see figure 3. The degree of change was analysed per patient. There was a median increase of 4 points in SGRQ score from baseline to start of exacerbation. There was a median reduction of 2 points in SGRQ score from start to end of exacerbation - figure 4.

Figure 3.

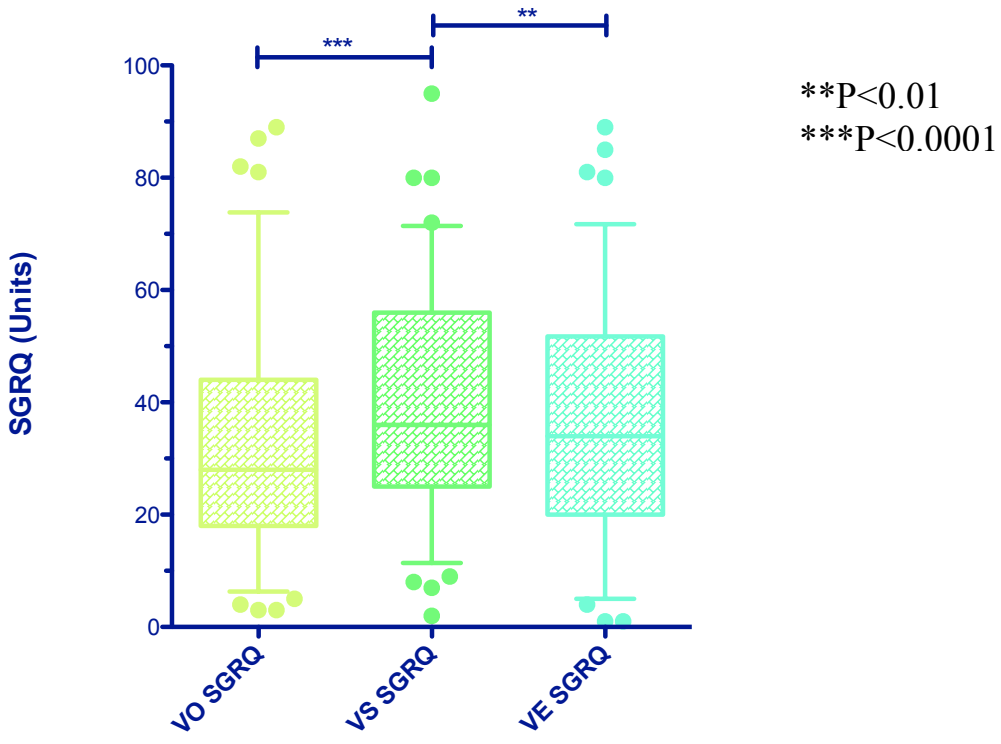
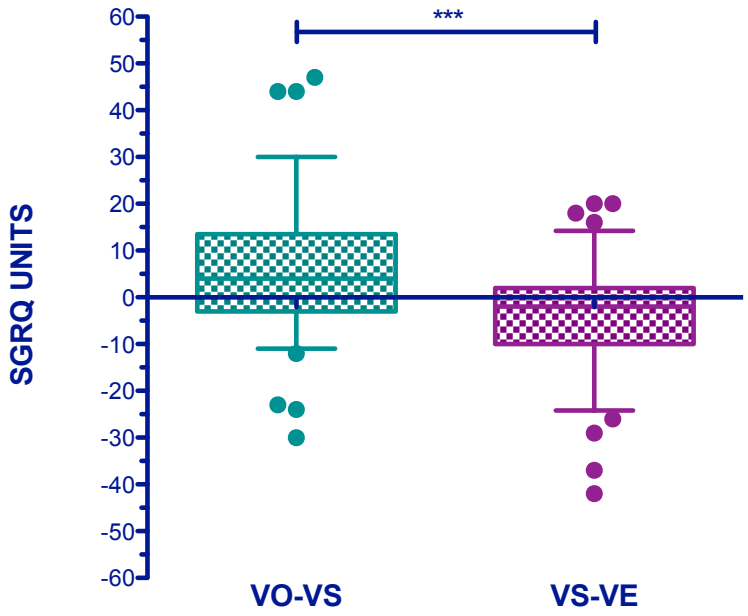


Figure to show the median value (IQR) St George's Respiratory Questionnaire (SGRQ) scores as baseline (VO), start of exacerbation (VS) and end of exacerbation (VE). Wilcoxon analysis **p<0.01, ***p<0.0001.

Figure 4.

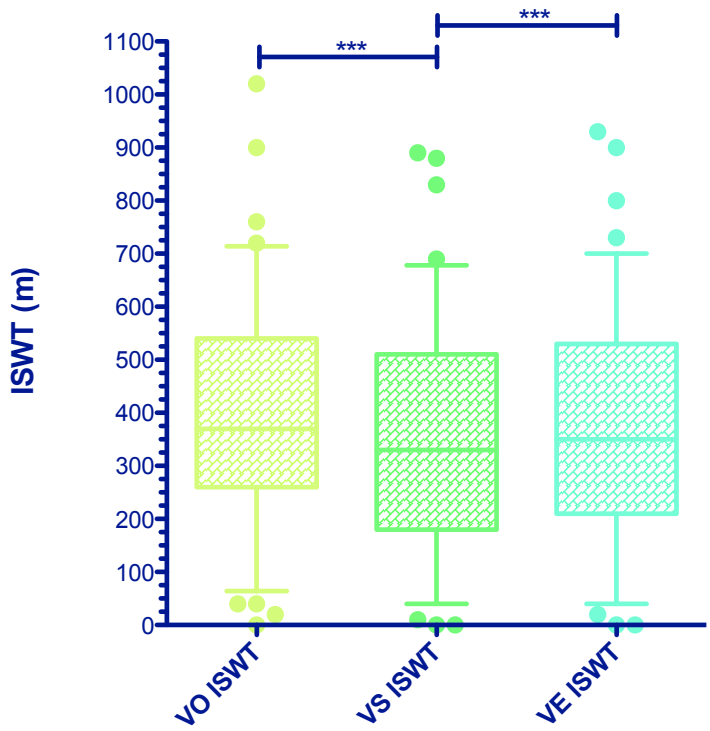


***P<0.0001

Figure to show the actual change in St George's Respiratory Questionnaire (SGRQ) value (units) from clinically stable bronchiectasis to start (VO-VS) and then from start to end of exacerbation (VS-VE).

Similarly, the ISWT median distance deteriorated from baseline to start of exacerbation and then improved following antibiotic treatment. Median values for distance walked at baseline (370m), start of exacerbation (330m) and end of exacerbation (350m) are shown below. There was a reduction of 40m walked from baseline to start of exacerbation. Patients were then given 14 days of antibiotics and the median distance walked improved by 50m. See figures 5 & 6.

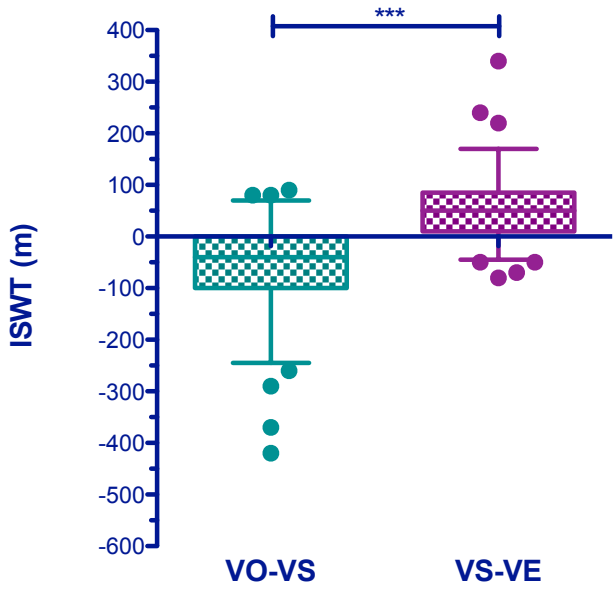
Figure 5.



***P<0.0001

Boxplot of median (IQR) with whiskers 5-95% percentiles of distance walked at baseline (VO), start of exacerbation (VS) and end of exacerbation (VE) in 94 patients. ISWT: incremental shuttle walk test.

Figure 6.

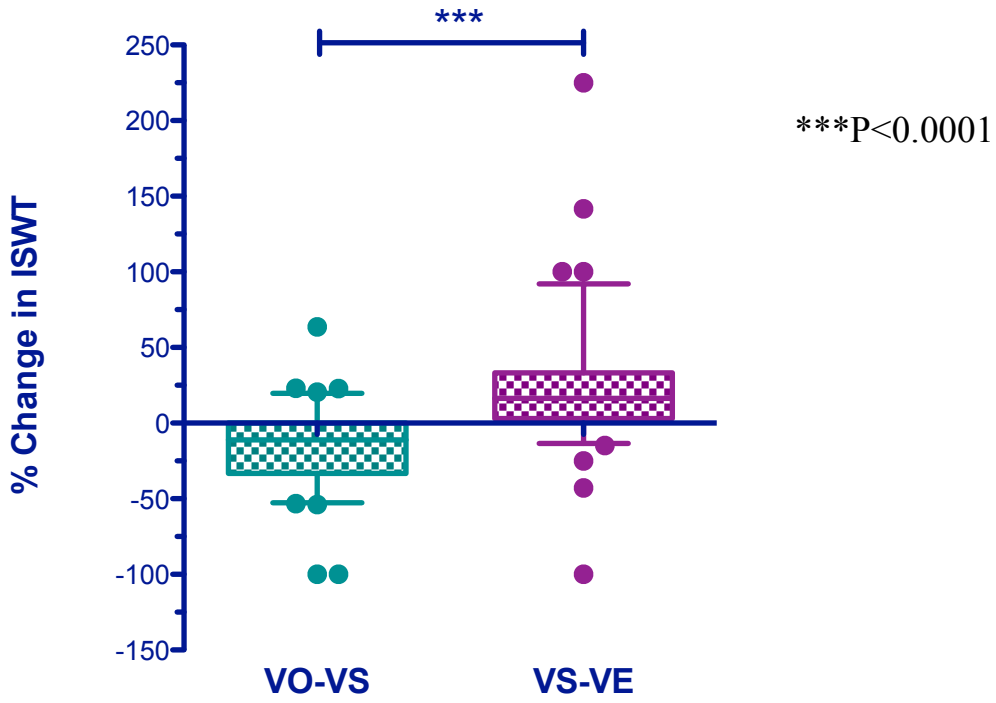


***P<0.0001

Figure to show the median (IQR) change with 5-95 percentile whiskers (m) in incremental shuttle walk (ISWT) distance walked with exacerbation (VO-VS) and after treatment (VS-VE). Wilcoxon analysis, ***P<0.001.

ISWT distance can be variable and is dependent on many factors including physical fitness. The percentage change in ISWT was analysed and found to drop by a median value 11.1% from baseline to the start of exacerbation. After 14 days of oral antibiotics, it improved by 16.3% (see figure 7).

Figure 7.



Boxplot of median (IQR) percentage change (%) with 5-95% whiskers in incremental shuttle walk test (ISWT) from baseline to start of exacerbation (VO-VS) and from start to end of treatment (VS-VE). Wilcoxon analysis, ***p<0.001.

To further illustrate validity of the ISWT, the distance walked was correlated with actual SGRQ scores and then the change in ISWT was correlated with change in SGRQ score – see figures 8 & 9. The percentage change in ISWT was also correlated with change in SGRQ score (figure 10).

Figure 8.

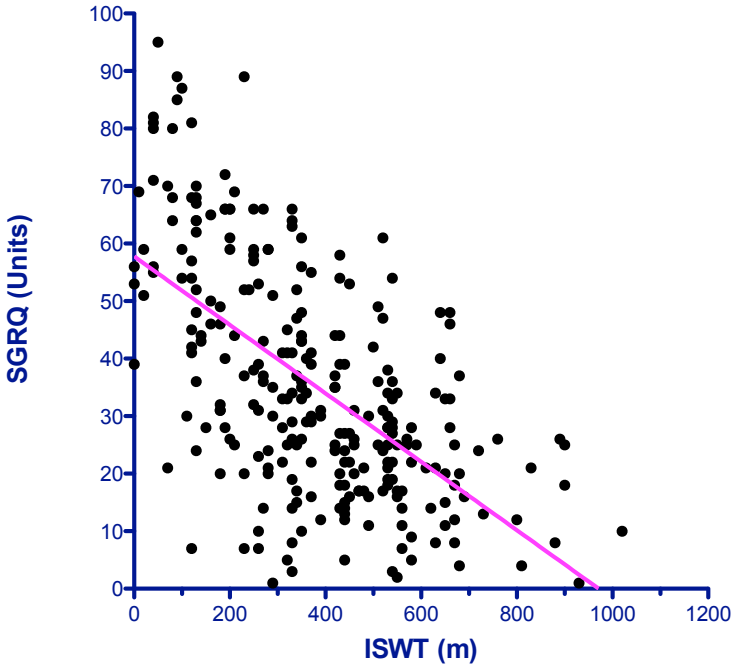


Figure to show the correlation between ISWT (m) and SGRQ scores in 282 events (94 patients at 3 different time points). R= ¯0.60, p<0.0001.

Figure 9.

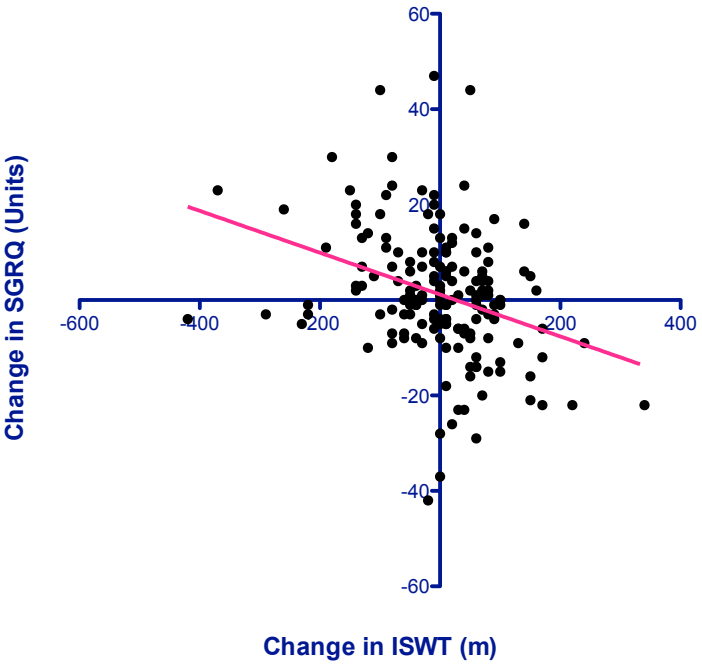
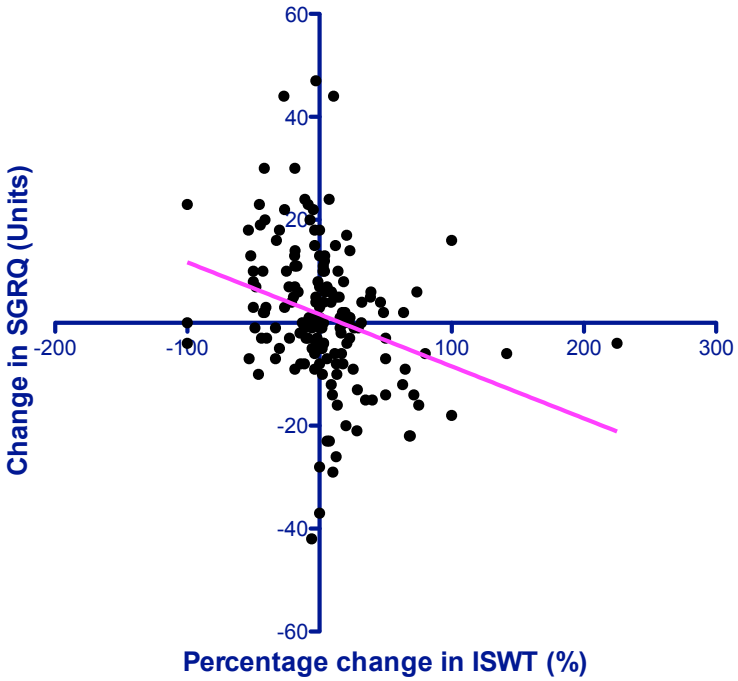


Figure to show the correlation between change in ISWT (m) and change in SGRQ scores (vo-vs and vs-ve), n=188. R=¯0.33, p<0.0001.

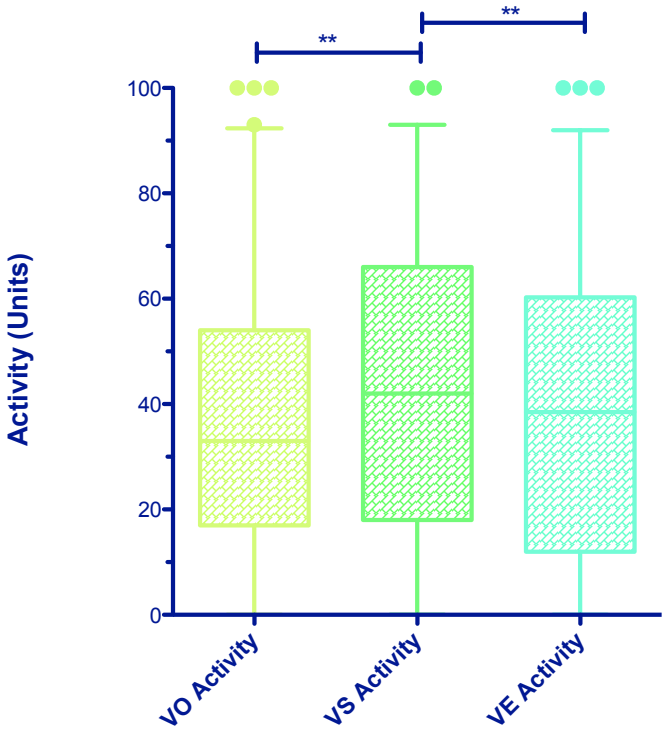
Figure 10.



Scatter plot to show the correlation between percentage change (%) in Incremental shuttle walk test (ISWT) and change in St George's Respiratory Questionnaire (SGRQ) scores from baseline to start of exacerbation (vo-vs) and start to end of exacerbation (vs-ve), n= 188. R=¯0.35, P<0.0001.

The 'Activity' component of the SGRQ changed with exacerbations: the median (IQR) values at baseline were 33 (17 - 54) to 42 (18 - 66) at visit start (p=0.002). The median then reduced to 38.5 (12 - 60.25) at visit end (p=0.009). The degree of change was analysed per patient. There was a median change of 0 (¯1 - 13) units in Activity score from baseline to start of exacerbation. There was a median reduction of 6 (¯14.5 - ¯6) units in activity score from start to end of exacerbation (p=0.002). See figures 11&12.

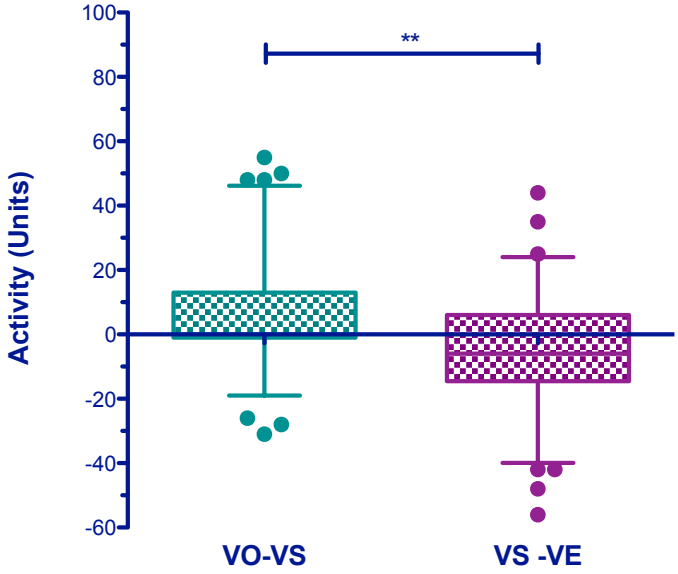
Figure 11.



**P<0.01

Figure to show the median (IQR) with whiskers 5-95percentile values for activity scores at baseline (VO), start of exacerbation (VS) and end of exacerbation (VE) in 94 patients with bronchiectasis. **p<0.01.

Figure 12.



**P<0.01

Figure to show the median (IQR) change in Activity component (units) with exacerbation (VO-VS) and after treatment (VS-VE). **p<0.01.

To further assess the validity of the ISWT, the distance walked was correlated with actual activity scores and then the change in ISWT was correlated with change in activity score – see figures 13&14. The percentage change in ISWT was also correlated with change in activity score (figure 15). Results were calculated using the Spearman's correlation.

Figure 13.

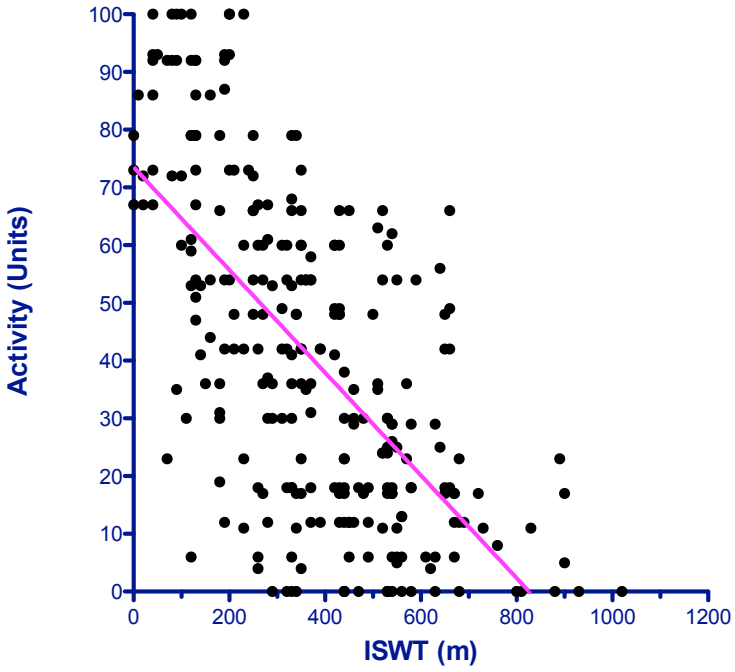


Figure to show the correlation between Activity scores and Incremental shuttle walk test (ISWT) in 94 patients at 3 different time points (n=282). R= -0.64, p<0.0001.

Figure 14.

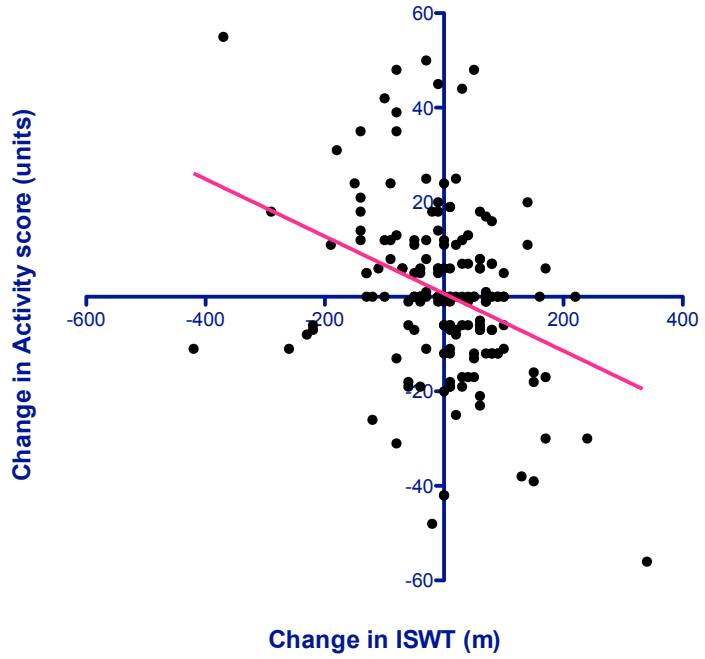


Figure to show the correlation between change in ISWT and change in Activity scores (vo-vs and vs-ve), n=188. R=-0.30, p<0.0001.

Figure 15.

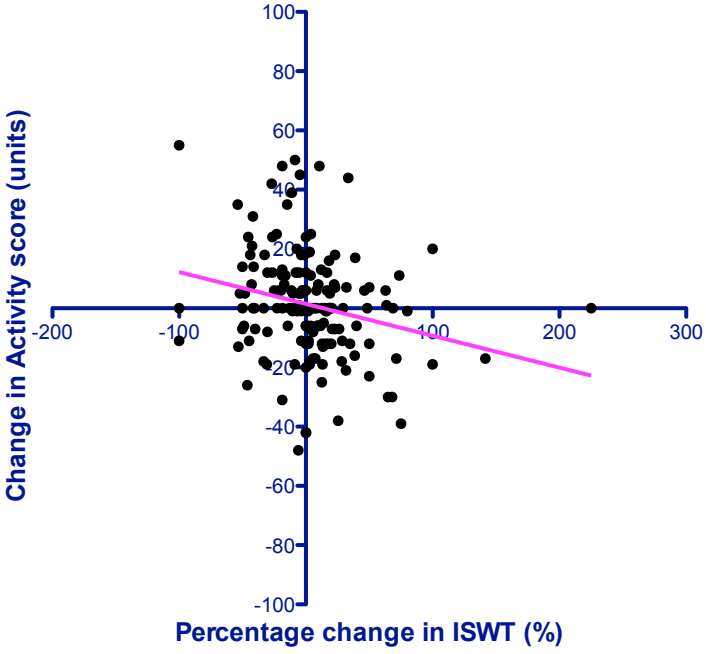
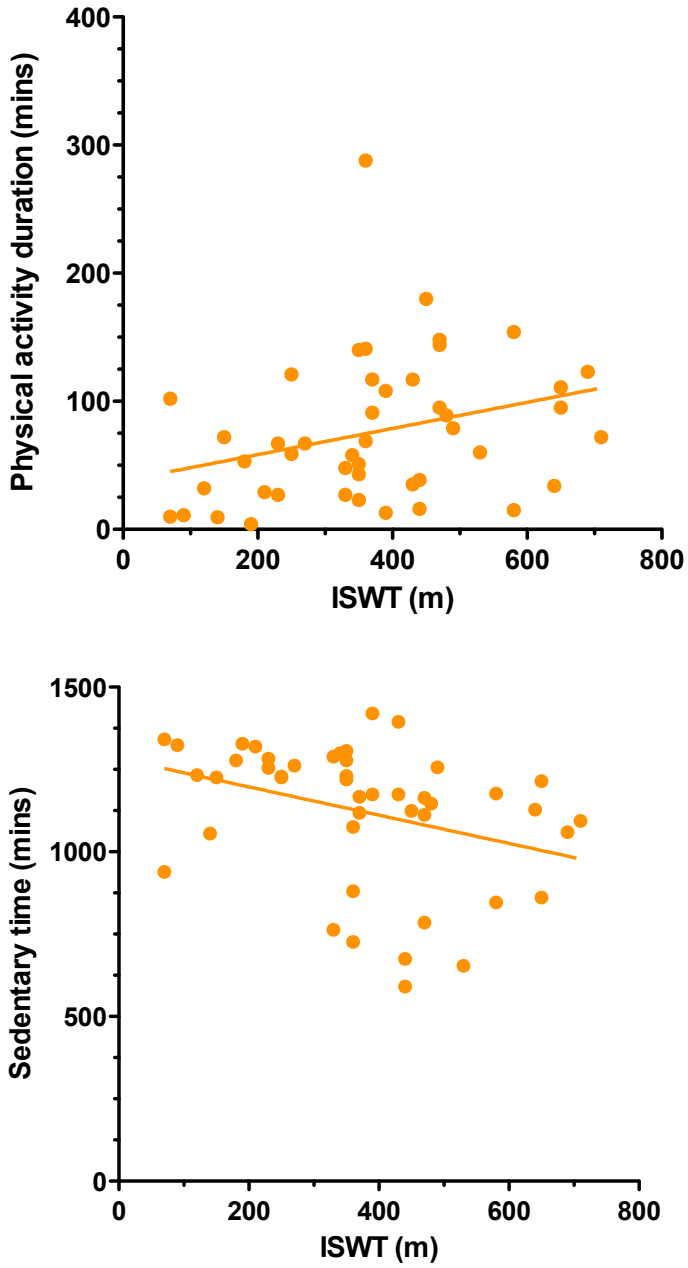


Figure to show the correlation between percentage change (%) in ISWT and actual change in activity scores from baseline to start of exacerbation (vo-vs) and start to end of exacerbation (vs-ve), n= 188. R=-0.27, P=0.0004.

A subset of patients were asked to wear an activity monitor for 1 week n=49. The average time spent being active and sedentary per day during this recorded time was correlated with ISWT distance walked at the start of the week. Correlations are shown below.

Figure 16.



Figures to show the correlation of Incremental shuttle walk test (ISWT) distance (m) with average physical activity duration (minutes) (above) and time spent sedentary (minutes) (below). R=0.42, p=0.004 and r=-0.48, p=0.0007.

Other parameters assessed include 'measured sleep' r=0.11, p=0.5; 'moderate activity' r=0.40, p=0.006; 'vigorous activity' r=0.13, p=0.4; 'measured active energy expenditure' r=0.35, p=0.02; 'number of steps' r=0.33, p=0.03 and 'Average metabolic equivalent (METs)' r=0.38, p<0.01.

To assess correlation with markers of severity the ISWT distance walked at each visit; baseline (VO), start of exacerbation (VS) and end of exacerbation (VE) was correlated with the MRC score for breathlessness recorded at each visit. Results showed a strong negative Spearman's correlation R=-0.59, p<0.0001.

ISWT scores at baseline were correlated with the bronchiectasis severity index (BSI) score calculated for each patient. Spearman's correlation was calculated to show R=-0.44, p<0.0001 (see below).

Figure 17.

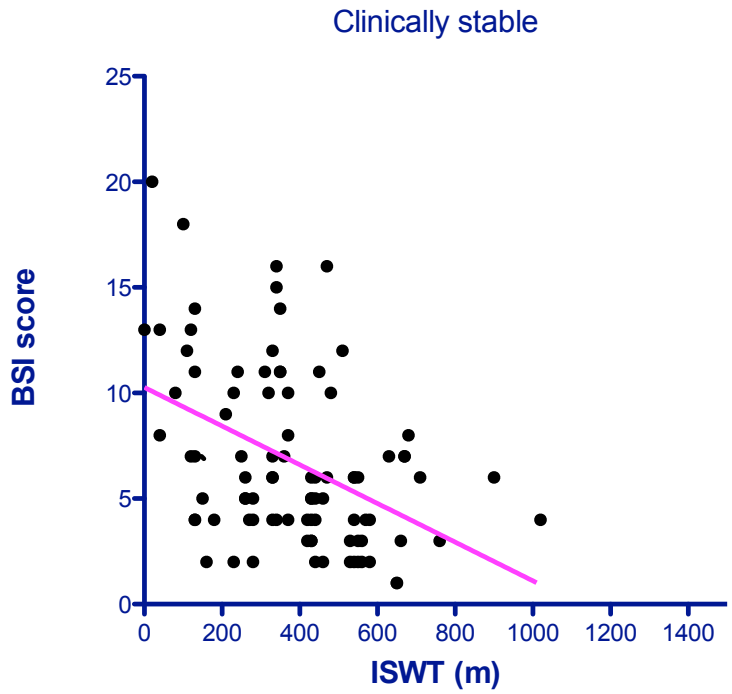


Figure to show correlation between incremental shuttle walk test distance (m) at baseline by 94 clinically stable patients with bronchiectasis with their corresponding Bronchiectasis Severity Index (BSI) score.

5.3.3.1 Responsiveness – Intravenous therapy

30 patients were given intravenous antibiotics for exacerbations of bronchiectasis as per the BTS guidelines (Pasteur *et al*, 2010). Their baseline characteristics are displayed below:

Table 3.

Characteristics	
Age yrs (mean, SD)	62.4 (11.4)
Female	47%
Smoking	70% never 30% ex-smoker 0% current
Inhaled/ oral corticosteroids	24/30 3/30
COPD	2/30
Asthma	13/30
Long-term antibiotics	3/30
Sputum microbiology	Pseudomonas 8/30 Other PPM 18/30 Mixed normal flora 5/30

Table to show the baseline characteristics of 30 patients with bronchiectasis including gender, smoking, medications, comorbidities and microbiology. PPM: Other potentially pathogenic microorganisms.

Patients were assessed at the beginning and end of intravenous therapy to ensure that improvement with intravenous therapy had occurred. The table below demonstrates an improvement in sputum colour assessed as per colour chart (Murray *et al*, 2009) and sputum volume, blood inflammatory markers and sputum bacterial clearance.

There was an improvement in FVC and %predicted FVC but no difference in FEV_1 or %predicted FEV_1.

Table 4.

	Day 0	Day 14	P value
Sputum colour	3.7	2.1	<0.0001
24hr volume	26.8mls	6.5mls	<0.0001
FEV_1 (L)	1.58	1.65	0.09
%FEV_1 pred.	56.6%	59.3%	0.1004
FVC (L)	2.53	2.84	0.002
%FVC pred.	72.7%	81.1%	0.0009
WCC $x10^9$/L	10.1	7.8	0.0004
ESR $x10^9$/L	29.1	22.2	0.0026
CRP (units)	22.5	4.7	<0.0001
PA	8/30	2/30	0.0003
Other PPMs	18/30	1/30	0.0001
MNF	5/30	17/30	0.0001
No growth	0	6/30	0.0001
yeasts	0	2/30	0.0210

Table to show biochemical, microbiological and clinical response to 14 days intravenous antibiotic therapy. FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, WCC: white cell count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, PA: *Pseudomonas aeruginosa*, PPMs: potentially pathogenic miceoorganisms, MNF: mixed normal flora.

At the start of exacerbation, patients were walking a median distance of 265m. After 14 days of intravenous therapy, the overall median distance walked improved by

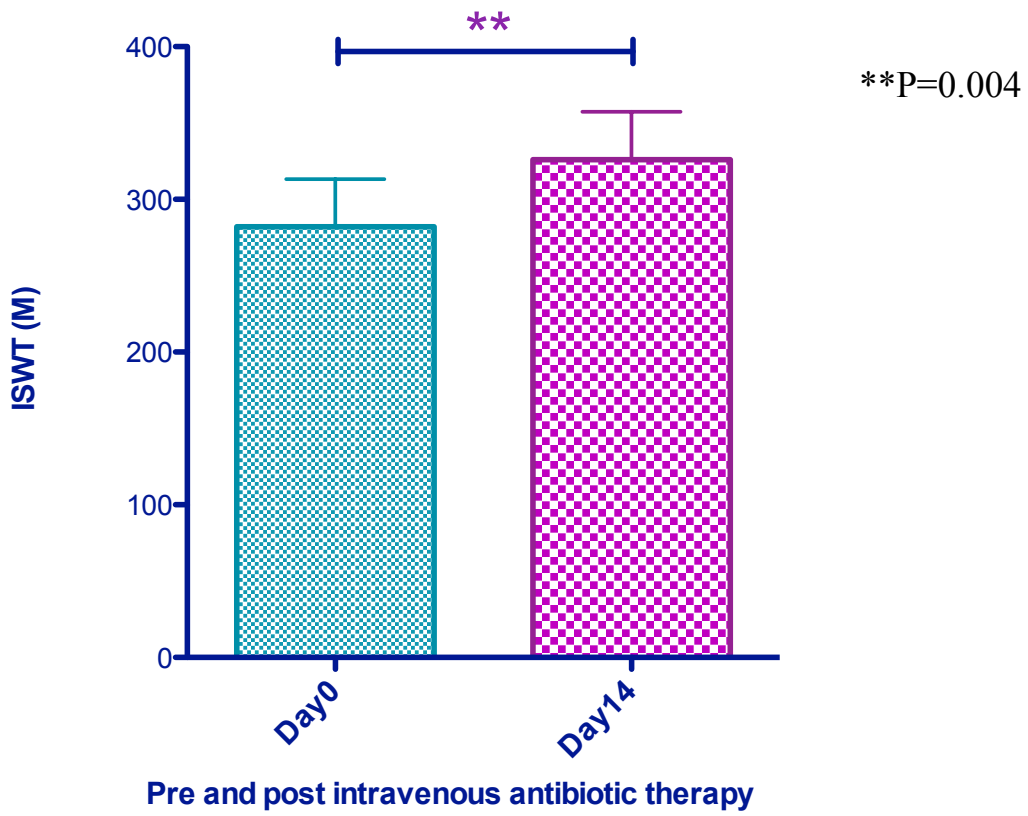
70m to 335m (see table 5 and figure 18). The median change per patient after 14 days of IV antibiotics was 32.5m (-12.5-90) and percentage improvement was 11.9% (-3.7 – 38.9%).

Table 5.

Value	Day 0 distance (m)	Day 14 distance (m)	Change in distance (m)	% change (%)
25% Percentile	137.5	190.0	-12.5	-3.7
Median	265.0	335.0	32.5	11.9
75% Percentile	395.0	450.0	90	38.9

Table to show distance walked pre- and post- intravenous antibiotic therapy.

Figure 18.



Barchart of mean (SEM) of Incremental shuttle walk test (ISWT) distance (m) pre and post 14 days of intravenous therapy. Wilcoxon analysis, **p<0.01.

5.3.3.2 Responsiveness - Oral therapy

94 patients attended for exacerbations that were managed on an outpatient basis with oral antibiotics. To ensure the patient had a genuine exacerbation, they were clinically assessed when stable and then again when developing an exacerbation – both before treatment was initiated and after completion. Baseline characteristics of the 94 patients are shown below.

Table 6.

Age	65.6 (10.7)
Female	54%
Smoking	62% never 36% ex-smoker 2% current
Inhaled/ oral corticosteroids	70% 2%
COPD	14%
Asthma	49%
Long-term antibiotics	7%
Sputum microbiology	Pseudomonas 13% Other PPM 76% Mixed normal flora 20%

Table to show baseline characteristic of 94 patients with bronchiectasis. COPD: chronic obstructive pulmonary disease, PPM: potentially pathogenic microorganisms.

To ensure patients had responded to oral therapy, various clinical parameters were measured both before and after treatment. The table below shows that after 14 days of oral therapy patients had improved inflammatory markers, lung function, sputum

production and increased microbial clearance of potentially pathogenic organisms and more culture of mixed normal flora.

Table 7.

	Day 0	Day 14	P value
Sputum colour	2.6	2.0	<0.0001
24hr volume	13mls	8.7mls	0.01
FEV_1 (L)	1.90	1.96	0.0009
$\%FEV_1$ pred.	75.8%	77.7%	0.007
FVC (L)	2.85	2.97	<0.0001
%FVC pred.	91.4%	94.9%	0.002
WCC $x10^9$/L	9.4	7.3	0.0003
ESR $x10^9$/L	25.6	19.6	0.01
CRP (units)	29.9	8.5	<0.0001
PA	12/90	12/90	1.0
Other PPMs	71/90	31/90	0.0001
MNF	19/90	37/90	0.004
No growth	0/90	10/90	0.001
yeasts	0/90	1/90	0.3

Table to show clinical markers at start and end of oral antibiotic therapy in 94 patients treated for an exacerbation of bronchiectasis. FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, WCC: white cell count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, PA: *Pseudomonas aeruginosa*, PPMs: potentially pathogenic miceoorganisms, MNF: mixed normal flora.

Patients walked a median distance of 370m on the incremental shuttle walk test when clinically stable at the baseline visit. At start of exacerbation the median distance walked was 330m (P<0.0001). After oral antibiotic therapy, prescribed according to available previous microbiology results, the median distance improved to 350m (P<0.0001). The distance walked at baseline did not differ from the distance walked after oral therapy, suggesting patients may have returned to their baseline level of function (see table 8 & figure 5). The median change (IQR) in distance walked from VO to VS was -40m (-100 – 0) with a median percentage reduction of 11.1% (-33.1% - 0%). The median change from VS to VE was 50m (10 – 85) with a median percentage improvement of 16.3% (3.1% – 33.3%).

Table 8.

Value	VO	VS	VE
25% Percentile	252.5	180.0	210.0
Median	370.0	330.0	350.0
75% Percentile	540.0	510.0	530.0

Table to show the ISWT distance walked pre- and post- oral antibiotic therapy.

5.3.3.3 Responsiveness - Nebulised therapy

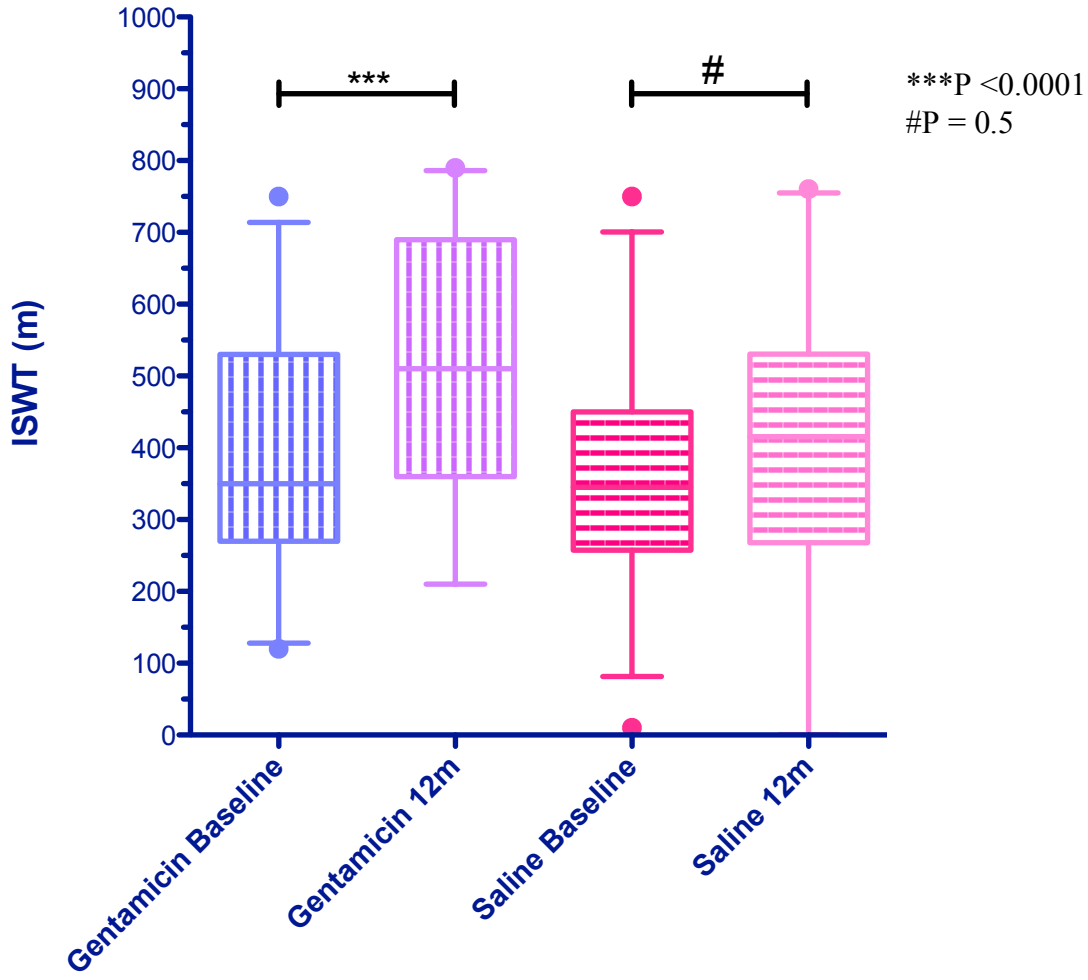
30 patients were randomly assigned to nebulised gentamicin therapy or placebo (nebulised saline). They performed an incremental shuttle walk test at baseline and repeated it at 12months of therapy. Median distances walked at each time point are displayed in the table below. The median difference (IQR) walked between baseline and 12months for the Gentamicin group was 70m (40 -160m) with a percentage median improvement (IQR) of 18.5% (11.1 – 45.7). There was a marked improvement in the Gentamicin group compared with the saline group with a mean difference of 90.4m (95% CI 40.76 – 140m, p=0.0006) and mean % difference 34.7% (95% CI 12.56 – 56.79, p=0.003) table 9 and figure 19.

Table 9.

Value	Nebulised Gentamicin		Nebulised saline	
	Baseline	End of therapy	Baseline	End of therapy
25% Percentile	270.0	360.0	257.5	267.5
Median	350.0	510.0	345.0	415.0
75% Percentile	530.0	690.0	450.0	530.0

Table to show the difference in distances walked (median, IQR) after 12months of either Gentamicin or placebo therapy (0.9% saline).

Figure 19.



Boxplots (median, IQR with 5-95 percentile whiskers) of Incremental shuttle walk test (ISWT) distance (m) improvement with nebulised Gentamicin b.d. therapy but not with nebulised 0.9% saline. ***p<0.001, #p>0.05.

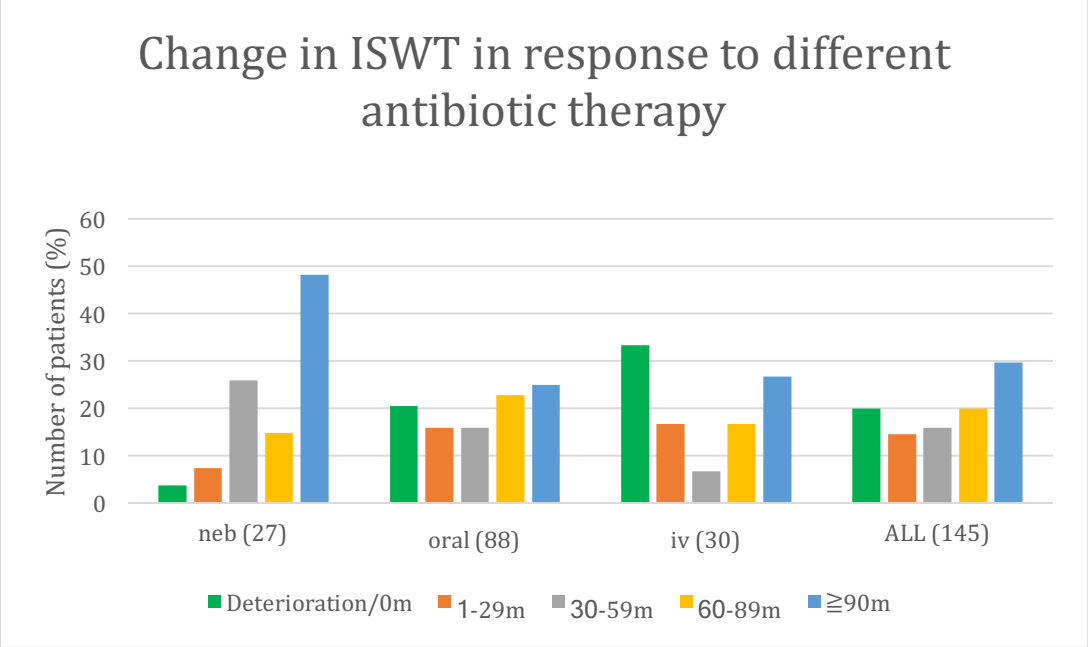
5.3.4 ISWT threshold for use as an objective clinical endpoint

The minimum clinical important difference (MCID) has been found to be 35m in a small study conducted in patients with bronchiectasis (Lee *et al*, 2014c). Our study measured ISWT distance walked in completion of increments of 10 metres. Therefore, in order to clarify what threshold of ISWT improvement is required to define a positive response we looked at the percentage of patients that had different metre and percentage improvements. We then investigated to see what level of improvement correlated best with clinically significant improvements in SGRQ score (4 or more unit improvement in overall score).

3.7% of those on long term nebulised therapy, 20.5% on short term oral antibiotic therapy and 33.3% on short term intravenous antibiotic therapy for acute exacerbation had deteriorated or had 0m improvement in ISWT distance. The trend is understandable as the sickest of patients would have been requiring 2 weeks of intravenous antibiotic therapy. 7.4% on nebulised, 15.9% on oral and 16.6% on intravenous antibiotics had an improvement between 1-29m. 25.9% of patients on nebulised therapy, 15.9% on oral therapy and 6.7% of patients on intravenous therapy had a 30-59m improvement. 14.8% on nebulised therapy, 22.7% of those on oral therapy and 16.7% on intravenous improved between 60-89m. 48.1% of patients on 12months nebulised therapy had a 90m or more improvement. 25% and 26.7% of patients on oral and intravenous respectively had a 90m or more improvement – see figure 20.

If a threshold of 30m was used then this would have included 65.5% of all the patients (on nebulised, oral and intravenous therapy). If a threshold of 60m was used, then this would include 50% of all patients.

Figure 20.



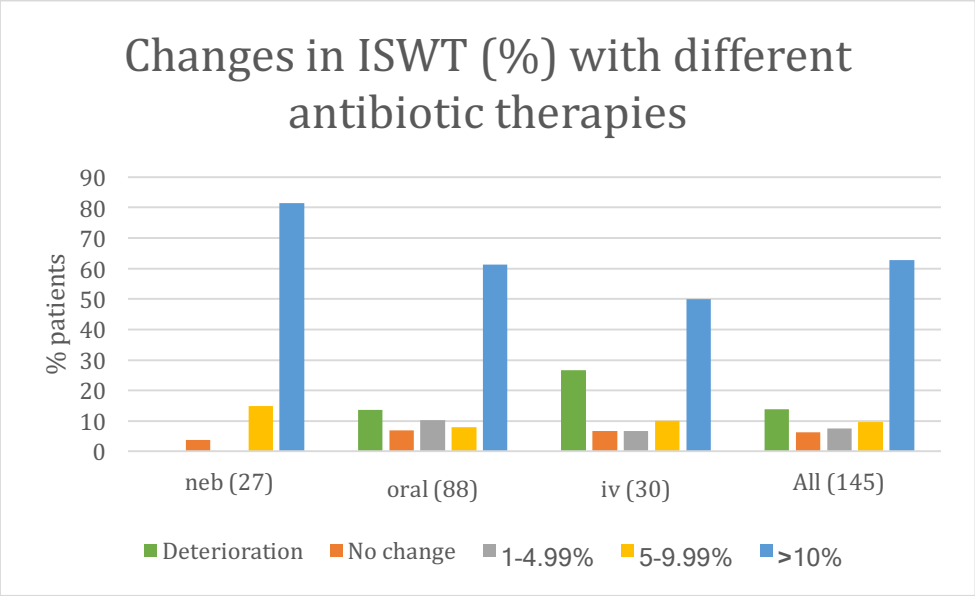
Barchart to show the percentage of patients with different metre incremental shuttle walk test (ISWT) improvements groups by medical therapy; nebulised, oral, intravenous and all therapies together.

Patients have different baseline functional capacity due to many factors incl. age, fitness, weight and comorbidities. An arbitrary metre improvement in ISWT might therefore underestimate improvement. To assess improvement whilst considering differing abilities, the percentage improvement in ISWT distance was investigated.

Figure 21 shows the different categories of improvement – 'deteriorated', 'no change', '1-4.99%', '5-9.99%' and '≥10%' improvement within the three treatment groups. There was no deterioration in the group receiving nebulised gentamicin twice daily. 13.6% and 26.6% or those on oral and intravenous antibiotic therapy respectively had a deterioration in the ISWT distance walked after 2 weeks of therapy. 3.7% of nebulised patients, 6.8% of oral and 6.6% of intravenous antibiotic patients had no change (0m) in the ISWT distance walked. Those that had a 1-4.99% improvement included 0% of nebulised patients, 10.2% of those on oral treatment and 6.7% of those in intravenous antibiotics. For patients on nebulised therapy 14.8% had a 5-9.99% improvement and 81.5% had a more than 10% improvement. Out of the patients on oral antibiotics, 8% and 61.3% had a 5-9.99% and >10%

improvement respectively. For patients on intravenous therapy 10% had a 5-9.99% improvement in ISWT distance and 50% had a ≥10% improvement.

Figure 21.



Barchart of the percentage of patients with different percentage improvement in incremental shuttle walk test (ISWT) by medical therapies.

5.3.5 Correlation of ISWT improvement with clinical improvement in SGRQ score

The minimum clinically important difference in SGRQ score is defined as 4 or more units. To further assess what the threshold of ISWT improvement is required to show when patients had clinically improved, we looked at the proportion of patients that had clinically significant improvements in SGRQ scores with therapy. 110 patients that had completed 14days of oral antibiotics or nebulised Gentamicin therapy also completed the SGRQ both pre- and post- treatment. Figure 22 shows the percentage of patients with a 4 or more unit rise in SGRQ scores with different categories of metre improvement in ISWT. If a threshold of 30m was taken to be an arbitrary cut off level, then 13.2% of all patients assessed that improved more than 30m also had a clinically significant improvement in SGRQ score. If a threshold of 60m was taken as the threshold, then 16.3% of these patients had a significant improvement in quality of life. In each metre improvement group, there were significantly more patients that did not have a significant improvement in SGRQ score than those that did.

Figure 22.

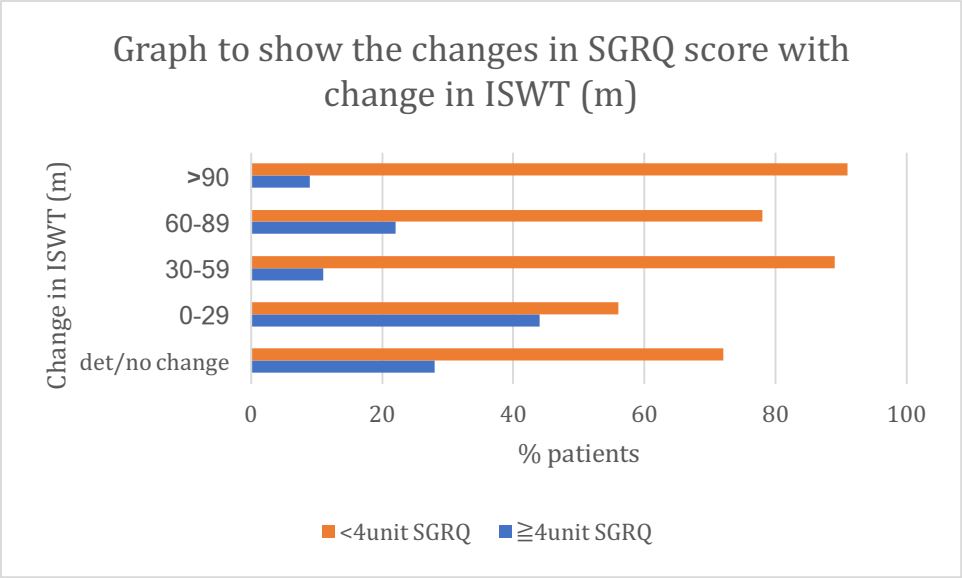


Figure to show the percentage of patients with a 4 or more unit rise in St George's Respiratory Questionnaire (SGRQ) score after antibiotic therapy within each incremental shuttle walk test (ISWT) metre improvement group; deterioration/no change, 0-29m, 30-59m, 60-89m and ≥90m.

Figure 23 shows the percentage of patients that had a 4 or more unit improvement in SGRQ score when patients were separated by percent improvement in ISWT after antibiotic therapy. The figure shows more patients had significant improvements in SGRQ score in the groups 5-9.99% and ≥10% than those who did not – 60%:40% and 62.5%:37.5% respectively. If a threshold of 5% improvement in ISWT distance walked after antibiotic therapy was used 62.2% of patients will also have significantly improved when measuring quality of life as assessed by SGRQ. If a threshold of ≥10% was used, then 62.5% of patients were captured that also reached the MCID for SGRQ improvement.

Figure 23.

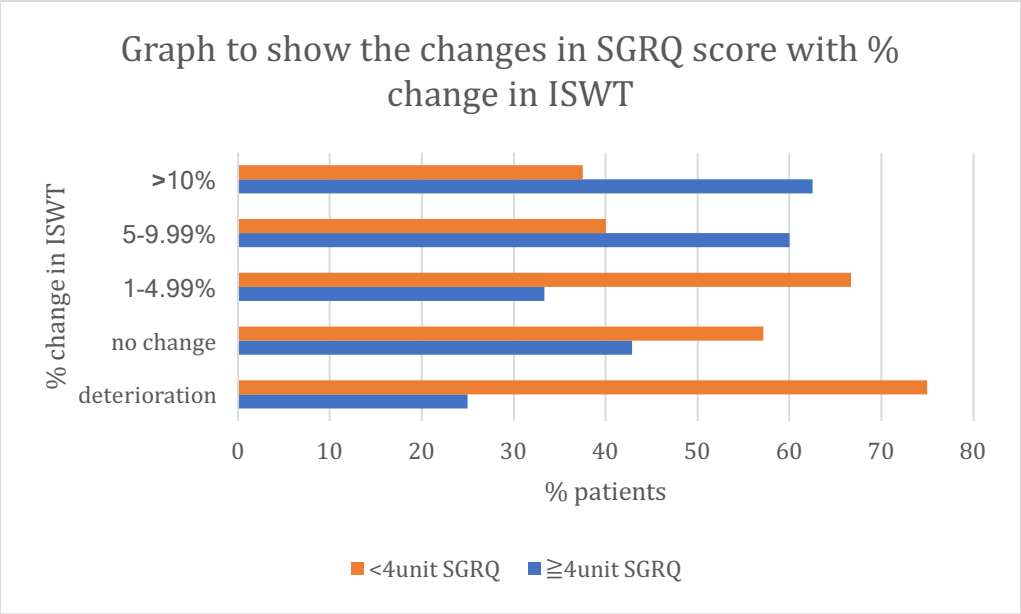


Figure to show the percentage of patients with a 4 or more unit rise in St George's Respiratory Questionnaire (SGRQ) score after antibiotic therapy within each incremental shuttle walk test (ISWT) percentage improvement group; deterioration, no change, 1-4.99%, 5-9.99% and ≥10%.

A clinically useful test would be when we could achieve at least 50% of patients having a 4unit or more improvement in SGRQ or more – see table 10. Using data from exacerbations, a 5% improvement or greater in ISWT would represent the MCID. Although all patients receiving antibiotic therapy improved, Erythrocyte Sedimentation Rate only improved in the group that had a 5% or more improvement in the ISWT ($p<0.0005$). Both groups had improved C reactive protein (change from Day 0 to day 14 after antibiotic in less than 5% improvement group $p=0.004$ and in more than 5% improvement group was $p<0.0001$), but the group that had 5% or more improvement had a greater reduction in C reactive protein, $p=0.007$.

Table 10.

% improvement in ISWT group	% of 110 patients	% with SGRQ improvement (≥4units) within the group	Average SGRQ change (Units)
Deterioration	10.9%	25%	-2.08
0.1-1%	6.4%	42.8%	-1.67
1.1-2.4%	1.8%	50%	-5.5
2.5% - 4.9%	6.4%	42.8%	5.29
5-9.9%	9%	60%	-8.28
10-19.9%	25.5%	53.6%	-6.86
≥ 20%	40%	68.1%	-9.86

Table 10: Percentage of patients with different percent improvements in ISWT, after treatment with oral and nebulised antibiotics for exacerbations (data not available in 14), with their average change in SGRQ score (a reduction of 4 or more units is a clinically significant difference).

The area under the curve for percentage ISWT change with a 4 unit improvement in SGRQ was calculated to be 0.79 (0.66-0.91, p=0.001). If a 2% improvement in ISWT was picked this had 92% sensitivity but 42% specificity. A 5% improvement had 92% sensitivity and 50% specificity. A 7.5% improvement had 88% and 58% respectively and a 10% improvement had 83% and 62% respectively.

5.4 Discussion

There has been an urgent need for an objective, validated clinical endpoint to assess response to existing and new therapies that prevent exacerbations and declining health status in patients with bronchiectasis. Current clinical endpoints routinely used in the management of patients with bronchiectasis each have their limitations. 24-hour sputum volume is thought to be inaccurate, unreliable and onerous. In clinical practice, this test is poorly complied with and difficult to interpret. Microbial clearance has been used to assess response to antibiotic treatment but often in clinical practice only qualitative microbiological analysis is performed and as patients are often chronically infected, this endpoint is difficult to interpret. Quantitative microbiological analysis has been carried out in phase 2 studies (Serisier *et al*, 2013b) but not for phase 3 studies to date (Haworth *et al*, 2014). In addition, there is controversy whether there is a change of microbial load with antibiotics (Tunney *et al*, 2013). C-reactive protein (CRP) can be useful in patients with a significant systemic response to infection. The degree of inflammatory response, however is highly variable among patients (Murray *et al*, 2009c). As such, it has not been used to assess longitudinal decline in bronchiectasis or any long-term therapies for bronchiectasis. Several quality of life questionnaires have been validated for use in bronchiectasis; SGRQ (Wilson *et al*, 1997b), LCQ (Murray *et al*, 2009d), Bronchiectasis health questionnaire (Spinou *et al,* 2017) & the Quality of life questionnaire-bronchiectasis (QOL-B) (Quittner *et al*, 2014 Quittner *et al*, 2015) but all are subjective outcome measures with patient self-assessment and reporting. The questionnaires can be cumbersome for the patient to complete and time-consuming for the clinician to analyse. For phase 3 studies, time to first exacerbation or mean exacerbations is used (Fan *et al*, 2015, Wong *et al*, 2012, Serisier *et al*, 2013). There has been debate about the definition of an exacerbation. A European Consensus statement has now been published that defines an exacerbation for use in clinical research (Hill *et al*, 2017).

Previous research by Carmargo and colleagues in patients with bronchiectasis has shown the ISWT to be reliable and represent functional capacity. 75 patients underwent cardiopulmonary exercise testing (CPET), two ISWT 30 minutes apart

while physical activity levels (steps per day) and dyspnoea score were recorded. ISWT significantly correlated with peak load (r=.82), VO_2 (r=.72), steps per day (r=.61) and dyspnoea (r=.69). Greater desaturation was achieved with ISWT than with CPET (Carmargo *et al*, 2009).

The incremental shuttle walk test has previously been used in studies of patients with bronchiectasis to assess the effect of interventions e.g. chest physiotherapy (Murray *et al*, 2009b) and pulmonary rehabilitation (Mandal *et al*, 2012, Newall *et al* 2005) but to date it has not been validated for use as an objective clinical endpoint in bronchiectasis. The main findings of this study support the ISWT to be reliable, valid and additionally, responsive to therapy (both short-term and long-term) in a large cohort of patients with bronchiectasis and therefore supports its use as an objective clinical endpoint.

The reliability was assessed over a 6-month period as the proposal would be to use the ISWT to assess both short or long-term therapies. At each time period, patients were stable and had not required antibiotic therapy for at least 28 days. The ISWT was found to be repeatable, in line with previously reported findings (Alves de Camargo *et al*, 2014).

The validity of the ISWT was supported with good correlations with both the total and activity SGRQ scores. The SGRQ was chosen as it is already validated for use in bronchiectasis and specifically measures activity as one of its domains. The correlation would never be perfect as the ISWT is an objective measure of exercise capacity whilst the SGRQ is a self-filled questionnaire but despite this remains strong with an R value of -0.6 ($P<0.0001$). In a sub group, there was a moderate correlation with sedentary and physically active duration time measured by activity monitors and further supports the proposal that the ISWT correlates well with other markers of exercise capacity. Overall there has been demonstration of at least moderate validity with the ISWT.

Disease severity can provide important prognostic information and the ISWT was found to moderately correlate with the Bronchiectasis Severity Index which might suggest its use in measuring severity although we acknowledge there are other factors that would influence a patient's ISWT score (e.g. fitness level, weight, comorbidity etc.) and therefore suggest serial measurements to show improvement or decline in ISWT score are more valuable than a one-off result. Furthermore, patients will all have differing baseline functional capacity so when the response to different treatments (short-term oral, short-term intravenous and long-term nebulised) was assessed the change in metres and percentage change was analysed.

This study used the response to therapies known to work in bronchiectasis and included patients treated with oral antibiotics and a more severe group receiving intravenous antibiotic therapy. Objective assessments showed patients did improve with short term antibiotic therapy and similarly there were significant improvements in ISWT. The third group chosen was the previously published study assessing the effectiveness of nebulised gentamicin over 1 year to assess the ISWT with effective long term therapy. Patients felt better with nebulised gentamicin therapy and there was a reduction in overall exacerbations compared with the group that received nebulised 0.9% saline therapy. There was similarly a significant improvement in the ISWT (in the gentamicin arm) over 1 year (Murray *et al*, 2011).

This study assessed to see whether there was a threshold of improvement in ISWT that could be used for future studies. For at least a 30m improvement, overall 66% of patients had met this improvement but if a higher threshold of at least 60m improvement was chosen, overall 50% met this improvement. Considering the overall biochemical and microbiological improvement it was difficult to appreciate that 50% of patients had not improved. A percentage improvement may however be more reliable and would take account of the variable baseline ISWT scores and functional capacity of patients. If a threshold of at least 5% improvement in ISWT is used, this would capture 73% overall (60% of those that received intravenous antibiotics, 69% of those that received oral antibiotics for 2 weeks and 96% of those that received inhaled gentamicin for 12m). If a threshold of at least 10%

improvement in ISWT is used, this would capture 63% overall (50% of those that received intravenous antibiotics, 61% of those that received oral antibiotics for 2 weeks and 81% of those that received inhaled gentamicin for 12m). Furthermore, both 5% and 10% improvement in ISWT groups had much higher percentage of patients with a clinically significant improvement in SGRQ outcomes (62.2% and 62.5% of patients with 4 or more unit improvements in SGRQ respectively) to further imply a percentage improvement that accounts for baseline function captured a group of patients that had truly clinically improved as opposed to using an arbitrary metre improvement with low numbers of patients that had met the minimum clinically important difference in SGRQ score. A percentage improvement in ISWT is a more reliable method of predicting clinical improvement than an arbitrary distance improvement. Using at least a 5% improvement in score as this captured 73% of our total cohort of patients, where the disease severity was mixed.

The area under the curve showed that the % ISWT was at least moderate value in predicting a 4 or more-unit improvement in SGRQ. As the cut-off rose, the sensitivity fell and specificity improved. For the MCID, the authors proposed that at least 50% should have a 4 or more-unit improvement in SGRQ and using a threshold of 5% ISWT improvement this was achieved when treating exacerbations of bronchiectasis. The authors would therefore recommend a MCID of 5% change in ISWT to be used as an objective endpoint for assessing response to new and existing therapies.

There were limitations to the study that included the lack of a placebo arm when assessing the response to therapy. Whilst this could have strengthened the impact of the responsiveness of ISWT to therapy, it would not have been ethical to withhold an effective treatment. To provide a similar dual arm situation, the Gentamicin trial was reanalysed which included a placebo arm. Gentamicin was also shown to be an effective therapy with a concomitant improvement in ISWT over 12months (Murray *et al*, 2011). The patients included in each subsection had varying degrees of severity of bronchiectasis with milder patients in the reliability and oral antibiotic

substudies. There were more severe patients in the studies investigating the area under the curve MCID and in the response to intravenous and nebulised antibiotics. Therefore, the applicability of these findings may require further investigation in patients with very severe bronchiectasis and further external validation in multi-centred studies is needed.

Previous research by Carmarco and colleagues in patients with bronchiectasis has shown the ISWT to be reliable (Carmarco *et al*, 2014) and the incremental shuttle walk test has previously been used to assess the effect of interventions e.g. chest physiotherapy (Murray *et al*, 2009b) and pulmonary rehabilitation (Mandal *et al*, 2012, Newall *et al*, 2005) on patients with bronchiectasis but to date it has not been validated for use as an objective clinical endpoint in bronchiectasis.

In conclusion, this study has confirmed the ISWT to be reliable, valid and responsive to change in patients with bronchiectasis. At least a 5% improvement in ISWT would be a useful objective endpoint to assess therapies in bronchiectasis.

Chapter 6:
Discussion and future work

Bronchiectasis is a chronic respiratory condition, characterised by dilated airways which give rise to destruction of the mucociliary escalator, the build-up of stagnant mucus and subsequently infection and inflammation which further destroy the airways. It has comparatively little research investigating it compared to other respiratory conditions such as asthma, chronic pulmonary obstructive disease and lung cancer. However, strides in bronchiectasis research have been made in the last two decades.

There is a multifactorial cause for its increasing incidence including; the increased use of the more commonly accepted diagnostic tool of computer tomography scanning which is the gold standard imaging modality for bronchiectasis; the increased awareness of distinction between bronchiectasis and other chronic lung conditions and the fact the ageing population are experiencing more hospital admissions and suffering from more respiratory infections like pneumonia which are a recognised cause for bronchiectasis. There is a recognised increased morbidity and mortality rate associated with bronchiectasis and this coupled with its increasing incidence makes bronchiectasis a respiratory condition of increasing research interest with a relative lack of evidence base for managing it.

There is a paucity of studies investigating patients with bronchiectasis that are largely managed in the community. Most new randomised controlled trials focus on cohorts with increased severity markers such as colonisation with *Pseudomonas aeruginosa*, poor lung function, repeated exacerbations and hospital admissions. Bronchiectasis is a progressive condition with newer therapies targeting the vicious cycle to prevent further progression of disease. It is therefore important to be manage the condition well, even in its less severe form.

This thesis aims to investigate patients with bronchiectasis that can be managed on an outpatient basis. The first chapter aimed to characterise these patients in their stable state before phenotyping their exacerbations in the clinically unstable state. The final chapter investigates validated endpoints in bronchiectasis which are vital to assessing treatment response in clinical practice as well as in research studies.

Recently, there have been two severity scoring systems developed to assess disease severity in bronchiectasis. The Bronchiectasis Severity Index score and the FACED score are two multidimensional scoring systems which categorise patients into low, medium and high severity brackets. The Bronchiectasis Severity Index (BSI) grades patients with bronchiectasis from a minimum score of 0 (very mild) to 26 (very severe) (Chalmers et al, 2016). The scores are classified into tertiles where 0-4 is a low score, 5-8 as intermediate and ≥9 is high score. The BSI incorporates 9 variables; age, body mass index (BMI), FEV_1 % predicted, hospital admission in the previous 2 years, number of exacerbations in the last year, MRC dyspnoea score, *Pseudomonas aeruginosa* colonisation, colonisation with other microorganisms and radiological severity. The BSI has been externally validated and shown to predict 30-day mortality and hospital admission rate and is also associated with the risk of future exacerbations and quality of life.

The FACED score is a slightly less complex scoring system incorporating only 5 variables including **F**EV_1 % predicted, **A**ge, Presence of chronic **C**olonization by Pseudomonas aeruginosa, radiological **E**xtension and **D**yspnoea. The overall sum of scores can range from 0 to 7 points where 0-2 points is classified mild bronchiectasis, 3-4 points is moderate bronchiectasis and overall 5-7 points is classified as severe bronchiectasis. The FACED score was later amended to a 9-point E-FACED score to include number of exacerbations in its severity scoring system.

Both scoring systems are comparable but the BSI was found to predict hospital admissions and differentiate the number of exacerbations and quality of life between the different severity tertiles.

The study in chapter one successfully recruited 208 patients that were clinically stable, i.e. no prescribed antibiotics or steroids for exacerbations in the last 6 weeks or any change to their regular respiratory medications. Of the 207 patients that continued with the study 74 had a BSI score 0-4, 79 had a BSI score 5-8 and 54 had a BSI score of 9 or above.

Each patients underwent a range of tests including quality of life questionnaires (The Leicester Cough Questionnaire and the St George's Respiratory Questionnaire), the incremental shuttle walk test, providing sputum for assessment of colour and 24 hour volume, sputum for quantitative bacteriological analysis, sputum for inflammatory marker assays of myeloperoxidase, neutrophil elastase and interleukin 8, blood for inflammatory serum analysis of primarily white cell count, neutrophil count, erythrocyte sedimentation rate and c-reactive protein and lung spirometry to assess forced expiratory flow in 1 second, forced vital capacity and mid expiratory flows with actual and % predicted values.

Results showed that increasing bronchiectasis severity index tertile correlated well with the LCQ assessing quality of life as a result of cough, the SGRQ assessing quality of life including activity and the incremental shuttle walk – a validated marker of functional capacity. There was also significant correlations of the BSI score with increased sputum colour (purulence), 24-hour sputum volume and bacterial load in sputum. Analysis of sputum colour and volume is quick and easy to assess and already part of routine practice. This thesis has investigated the importance of bacterial load but there is conflicting results in the literature and hence requires further analysis.

The bronchiectasis severity index score also significantly correlated with all serum inflammatory markers, sputum myeloperixodase and all measured parameters of lung function but not with sputum neutrophil elastase or sputum interleukin -8. The above clinical endpoints have been previously investigated and found to be clinical importance in bronchiectasis:

The Leicester Cough Questionnaire (LCQ) (range 3-21, 3 most severe cough severity) and the St George's Respiratory Questionnaire (SGRQ) (range 0-100, 100 most severe) have both been validated as clinical endpoints for use in bronchiectasis (Murray *et al*, 2009d Wilson *et al*, 1997b). The MCID for the LCQ is 1.3units and for the SGRQ is 4units.

The ISWT is an objective measurement of functional capacity and has been previously shown to remain stable in patients with clinical stability i.e. no change to medications and no exacerbation 6 months apart (Mandal *et al*, 2014). It has also been shown to improve with long-term nebulised antibiotics (Murray *et al*, 2011) and anti-inflammatory treatment (Mandal *et al*, 2014).

Sputum colour was reported to have good reliability between patients and clinician and found to be predictive of bacterial colonisation ($p<0.0001$). Independent factors associated with sputum purulence were found to be bacterial colonisation, radiological severity with varicose or cystic bronchiectasis, FEV_1 less than 80% predicted and diagnosis of bronchiectasis aged less than 45yrs old (Murray *et al*, 2009).

Chalmers and colleagues reported there was a direct relationship between bacterial load and the severity of exacerbations and risk of subsequent exacerbations. They linked bacterial load with airways inflammation and systemic inflammation when assessing both inpatients and outpatient exacerbations. Furthermore, they reported the reduction of bacterial load with short and long term antibiotic therapy (Chalmers *et al*, 2012).

Wilson and colleagues reported patients with bronchiectasis have raised inflammatory markers when clinically stable and that inflammatory markers correlate with disease severity; WCC, neutrophil count, ESR and CRP all correlated with radiological severity, WCC and CRP correlated with total SGRQ scores (Wilson *et al*, 1998).

Airway inflammation has been shown to correlate with higher bacterial loads and subsequent increased risk of exacerbation and with more severe exacerbations (Chalmers *et al*, 2012). Inflammatory markers have been reported to be increased in patients colonised with *Pseudomonas aeruginosa* and in those with more severe radiological bronchiectasis (Chalmers *et al*, 2012). Elevation of Neutrophil elastase was found to be associated with the bronchiectasis severity index score ($r=0.49$,

p<0.0001) but was not independently associated with mortality rates. The authors found neutrophil elastase to rise in exacerbations and have good discrimination for severe exacerbations and all-cause mortality (*Chalmers et al*, 2016). Airway inflammatory markers have been shown to improve with the use of long term antibiotic use (Murray *et al,* 2011 and Chalmers *et al*, 2012)

Lung function (50% or lower FEV_1) was associated with increased radiological severity (p<0.01), presence of *Pseudomonas aeruginosa* (p<0.01) and the presence of symptoms for 10 or more years (p=0.01) (Guan *et al*, 2014). Martinez-Garcia also found accelerated decline in FEV_1 to be associated with more severe exacerbations, the presence of *Pseudomonas aeruginosa* in sputum and systemic inflammation (Martinez-Garcia *et al*, 2007).

These results showed that in addition to the existing 9 variables included in the severity score, these other clinically relevant endpoints with the exception of inflammatory marker interleukin-8 might be valuable in assessing disease severity. The sample size was not large enough to investigate whether these clinical endpoints could also help predict mortality in this cohort but larger studies investigating these endpoints in conjunction with the Bronchiectasis Severity Index would be interesting to see if its predictive ability could be more sensitive although acknowledging this might make the scoring system more complex.

The next study investigated outpatient exacerbations of patients with bronchiectasis to further help phenotype this patient cohort. Patients were invited to call the dedicated clinical research fellow if they felt they were experiencing an exacerbation of their disease during normal working hours Monday to Friday, 9am to 5pm. They would be seen as soon as possible and undergo a series of tests before being issued with a 14 day prescription for antibiotics based on their previous sputum culture results. Patients then re-attended after completing the treatment course to repeat the same series of tests.

Results showed that all clinical markers including FEV_1 and FVC actual and predicted values, sputum colour, spontaneous and 24hour sputum volume and MRC dyspnoea scores all deteriorated when patients experienced an exacerbation. There was a 4 unit deterioration in LCQ score (MCID 1.3 units) and 4.5 units deterioration in SGRQ score (MCID 4 units) in keeping with literature that report exacerbations reduce quality of life.

This cohort of outpatient exacerbations, which by the virtue of being able to be managed on an outpatient basis could be classed as less severe all had significant rises in serum inflammatory markers (WCC, neutrophils, ESR and CRP). In addition, there was also evidence of significant increase in sputum inflammatory marker myeloperoxidase and a trend towards an increase in neutrophil elastase. All markers of inflammation improved with short term antibiotics for 14 days.

Quantitative microbiology results revealed culture of pathogens commonly associated with bronchiectasis including: *Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Enterobacter, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Proteus mirabilis, Acinetobacter baumannii, Achromobacter xylosoxidans, Aeromonas hydrophila, citrobacter freundii, Stenotrophomonas maltophilia, mixed normal flora,* and occasionally there was no growth. There was significantly more growth of pathogenic organisms and of multiple pathogens cultured by patients at the start of exacerbation when compared with baseline. 10% of culture results grew Pseudomonas aeruginosa at baseline and whilst this did not vary much at start of exacerbation (12%) and end of exacerbation (12%), there was significantly more growth of potentially pathogenic microorganisms and less mixed normal flora with exacerbation.

Interestingly, there was a 57% overall change in the dominant pathogen cultured at start of exacerbation with baseline culture results. The overall median bacterial count at baseline was 6.7×10^5 CFU/ml. This increases significantly by 2log units to 1.2×10^7 CFU/ml at start of exacerbation and returns to a median value of 0 CFU/ml

at the end of exacerbation visits. This 2-log unit rise in bacterial load is not seen if the dominant pathogen remains the same but only if it changes (p=0.02). There was a significant higher proportion of patients that had a 1 or more log rise in bacterial load if the dominant pathogen on sputum culture had changed then if the dominant pathogen had not changed (P=0.0007). This is the first study to demonstrate a change in dominant pathogen leads to a rise in bacterial load.

To further investigate the importance of a rise in bacterial load, patients results were sub analysed to detect any clinical differences between those that had a 1 or more log unit rise in CFU/ml in bacterial load at start of exacerbation and those that didn't. There were significantly more patients with a 10% or more reduction in FEV_1 and %predicted FEV_1 in the group with 1 or more unit rise in bacterial load (p=0.04), a significant increase in the volume of spontaneous sputum production on exacerbation in the higher bacterial load group (P=0.04) but this was not seen in the 24-hour collection of sputum production or change in sputum colour. There was an overall larger reduction in exercise tolerance in the group with 1 or more unit log rise in bacterial load (60m versus 30m) but this was not significant. However, there was significantly more patients with an overall 5% reduction in their exercise tolerance in the 1 or more log unit rise in bacterial load (74% versus 52%), p=0.049.

There was some evidence to support the presence of increased inflammation in those with a higher bacterial load count as has already been previously described in the literature (Chalmers *et al*, 2012) with patients in this cohort more likely to have a doubling of their white cell count (p=0.02) and an increase in 50% of their neutrophil count on exacerbation (p=0.04). There was also a significantly higher increase in myeloperoxidase and neutrophil elastase levels with a 1 or more log unit rise in bacterial load on exacerbation.

The respiratory symptoms that patients reported on start of exacerbation were well recognised symptoms of exacerbations with 91% reporting worsening cough, 78% reporting increased sputum volume, 72% reporting increased sputum purulence and 79% reporting worsening breathlessness. The group with a higher rise in bacterial

load were more likely to develop chest pain, have increased sputum volume and deny any headache. This symptom complex was more specific with a high positive predictive value for recognising a rise in bacterial load of 1 or more log units than either of the British Thoracic Society or European Respiratory Society consensus definitions of exacerbations but it lacked sensitivity.

Lastly, the effect of viruses on exacerbations of bronchiectasis was explored. Of the 94 outpatient exacerbations, 90 had a viral throat swab performed. 16 of these were positive for common recognised viruses such as rhinovirus and influenza. There was no significant difference in the number of viral positive samples between the higher ≥1 log bacterial CFU rise and < 1Log rise in bacterial CFU groups, p=0.6 suggesting viruses do not affect bacterial load in bronchiectasis exacerbations, although the number of positive viral samples were small in this cohort (18%).

These 94 outpatient exacerbations revealed deterioration in all clinical markers from baseline. All clinical markers improved with a 14 day course of antibiotics. Bacteriology results in this cohort demonstrated the change in microbial flora and bacterial load on the start of exacerbation in addition to the clinical importance of a demonstrable rise in bacterial load of 1 or more log units in CFU/ml. The way in which exacerbations of bronchiectasis is currently defined based largely on expert opinion of symptomology may not be the most accurate method to employ in a society where antibiotic guardianship becomes increasingly paramount. This study has demonstrated that there may be a case for more objective treatment of exacerbations, such as those employed when treating urinary tract infections; positive urine culture with bacterial load above a certain threshold coupled with classical symptoms. Further larger studies are required to explore the clinical consequences of an increase in bacterial load and the threshold at which colonisation becomes infection and requires treatment.

All clinical markers improved with a 14 day course of antibiotics. The optimum duration of antibiotic therapy has not been explored in large randomised controlled trials. Patients with bronchiectasis often have chronically colonised airways and

after repeated courses of antibiotics may have pathogens with multiple resistant profiles. These patients may require a longer course of antibiotics but in patients with common pathogens and a lower bacterial load signalling a less severe exacerbation may improve sufficiently with a shorter course of antibiotics. Randomised controlled trials are needed to assess the optimum length of treatment.

In order to assess response to treatment there needs to be robust validated endpoints to measure. The first two chapters showed how all the clinical markers assessed (sputum colour, spontaneous sputum volume, 24-hour sputum volume, lung function parameters, LCQ score, SGRQ score, ISWT score, serum markers of inflammation and sputum markers of inflammation) all correlated with bronchial severity index scores and deteriorated from baseline levels when patients experienced an exacerbation. Some of these endpoints have already been validated for use in bronchiectasis but each have their limitations.

Sputum colour is thought to be useful in assessing severity but it has not been validated for use as an endpoint. 24-hour sputum volume is poorly complied and therefore often inaccurate and difficult to interpret. Microbial clearance has been used to assess response to antibiotic treatment but would not be a suitable endpoint in patients that culture mixed normal flora and in those that are unable to expectorate. Qualitative analysis is not usually performed in clinical practice and has currently only been carried out in phase 2 trials (Serisier *et al* 2013b). Qualitative or quantitative microbial culture takes at least 24 hours to perform so results would not be readily available.

Serum inflammatory markers have not been validated for use as endpoints and they rely on patients mounting a significant systemic response to infection. As such, it may be of limited use in patients that are clinically stable. There has been some interest in sputum inflammatory markers such as neutrophil elastase which is considered to increase with bacterial load but again, none have been validated for use in bronchiectasis as a useful endpoint.

Several quality of life questionnaires have been validated for use in bronchiectasis; SGRQ, (Wilson *et al,* 1997b) LCQ (Murray *et al*, 2009d), Bronchiectasis health questionnaire (Spinou *et al,* 2017) & the Quality of life questionnaire-bronchiectasis (QOL-B) (Quittner *et al*, 2014 & Quittner *et al,* 2015). These are more subjective outcome measures incorporating patient self-assessment and reporting. The questionnaires can be cumbersome for the patient to complete and time-consuming for the clinician to analyse.

The results in the first two chapters showed some interesting findings with the incremental shuttle walk test. This is the only marker of functional capacity tested and hence can provide insight into how exacerbations can physically impact on the body. In the first chapter, the ISWT correlated well with the BSI, demonstrating reducing exercise capacity with increasing severity. In the second chapter, the ISWT deteriorated significantly with exacerbation and improved with treatment. In addition, a reduction in 5% of ISWT walk distance was associated with a 1 or more log unit rise in bacterial count CFU/ml. In this growing field of bronchiectasis research more validated clinical endpoints are required to fully assess existing and new therapies. Therefore, the third chapter assesses the incremental shuttle walk test as a validated clinical endpoint for use in patients with bronchiectasis.

In order to validate the incremental shuttle walk test the reliability, validity, and responsiveness of the test were investigated. For reliability, 30 patients completed the ISWT 6 months apart when clinically stable. There was no significant change in distance walked (390m versus 400m) over 6 months ($p=0.48$) and a Bland-Altman plot showed a mean bias of -1.38m with a calculated intraclass coefficient to be 0.85 ($p<0.001$).

Validity was assessed by correlating ISWT distance walked with total and activity components of the SGRQ score in 94 patients at three different time points (clinically stable, start of exacerbation and end of exacerbation, n=282). There was a reduction in ISWT distance walked at start of exacerbation and similarly there was an increase in SGRQ scores with exacerbation (the higher the score, the bigger the

impairment in quality of life). A Spearman's correlation between the two indices was highly significant, R=-0.60, p<0.0001. Similarly, the correlation between the activity component and ISWT distance walked was also highly significant, R= -0.64, p<0.0001.

A subset of patients were asked to wear an activity monitor for 1 week n=49. The average time spent being active and sedentary per day was recorded and shown to moderately correlate with ISWT; physical activity duration (R=0.42, p=0.004) and sedentary time (R=-0.48, p=0.0007). Further correlations with other markers of disease severity; MRC dyspnoea score (R=-0.59, p<0.0001) and bronchiectasis severity index score (R=-0.44, p<0.0001) were strong.

Responsiveness of the ISWT to intravenous, oral and nebulised therapy was demonstrated. 30 patients were given intravenous antibiotics for exacerbations of bronchiectasis as per the BTS guidelines (Pasteur *et al*, 2010). The patients showed overall clinical improvement and the ISWT improved from 2656m to 335m (p=0.004) with an overall median percentage improvement of 11.9% (-3.7 – 38.9%). 94 patients were treated with oral antibiotics for an exacerbation. The median change in distance walked from baseline to start of exacerbation was a significant reduction of 40m. After 14 days of oral antibiotics there was a significant median improvement in ISWT distance of 50m or 16.3%.

30 patients were previously treated with nebulised gentamicin or placebo (nebulised saline) for 12 months (Murray *et al*, 2011). Reanalysis of the results showed the median difference (IQR) for the Gentamicin group was 70m (40 -160m) with a percentage median improvement (IQR) of 18.5% (11.1 – 45.7). There was a marked improvement in the Gentamicin group compared with the saline group with a mean difference of 90.4m (95% CI 40.76 – 140m, p=0.0006) and mean % difference 34.7% (95% CI 12.56 – 56.79, p=0.003).

The minimum clinical important difference (MCID) has been found to be 35m in a small study conducted in patients with bronchiectasis (Lee *et al*, 2014c). As patients

can have different baseline functional exercise capacity, an arbitrary metre distance might under-report improvement in those with poor functional reserve and over report improvement in patients that have good cardiovascular reserve. A percentage improvement would take a patient's baseline capability into account. A 5% improvement in ISWT captured 73% of all patients that responded to some type of antibiotic therapy. There was also a higher proportion (62.2%) of patients that had a significant (more than 4 unit) improvement in SGRQ score in those that had a 5% or more improvement in ISWT.

The minimum clinically important difference was recommended to be a 5% improvement in ISWT as this had a moderate ability to predict a 4 or more unit improvement in SGRQ score. The ISWT is an objective test of functional capacity and a 5% improvement in distance walked has been demonstrated as a validated clinical endpoint for use in patients with bronchiectasis. Further studies assessing the external validation of a 5% improvement in ISWT would now be necessary before this clinical endpoint can be routinely used in clinical practice and research studies.

Future work

This thesis has characterised a cohort of bronchiectasis patients that are usually managed as outpatients in their clinically stable state and when unwell with an exacerbation of bronchiectasis. All exacerbations were managed as outpatients and so the phenotype of outpatient exacerbations including the clinical importance of assessing bacterial load in this cohort was further clarified.

Quantitative and qualitative microbiology was performed according to conventional microbiological culture techniques. DNA sequence based molecular methods have identified a complex and diverse polymicrobial community exists in the lungs but the clinical implications of such detailed assessment is not completely understood. The role of lung microbiota in the pathogenesis and progression of bronchiectasis remains poorly understood but this thesis highlights the potential of higher bacterial loads in the unstable, exacerbation state for a more severe phenotype of exacerbation. Further work examining the implication of higher bacterial loads in the stable state in the progression of disease is needed and whether antimicrobial therapy would benefit patients with high loads of colonising bacteria. In addition, further studies comparing conventional microbiological techniques with molecular microbiology techniques including 16S DNA is required to gain a better understanding of the comprehensive cross sectional analysis of bacterial isolation within the airways.

A 1 log unit rise or more in bacterial load was explored in this thesis as a significant increase in bacterial load that is known to have increased airway and systemic inflammation, however further work is required to determine the exact threshold of change that is clinically important in terms of differentiating, if possible, between colonisation and infection and between mild and severe disease.

The implications of bacterial load could be explored even further. Currently, the optimum duration of antibiotic treatment is unknown and a 14day course is recommended based on expert opinion in the British Thoracic Society guidelines. If, lower bacterial loads are predictive of less severe exacerbations, as implied by the work in this thesis, then a shorter course of antibiotics may be prudent in those with a lesser rise in bacterial load. Randomised controlled trials are required to explore this further.

The work contained in this thesis reported a change in dominant pathogen was causative of a 1 log unit rise in bacterial load on start of exacerbation. Currently, empirical antibiotic treatment for exacerbations is recommended based on any

previously available sputum microbiology results. This thesis suggests the way we treat exacerbations requires further investigation as empirical antibiotics based on previous microbiology might not work in all patients and highlights the importance of sending sputum for bacterial culture prior to starting empirical antibiotic therapy and of reviewing patients for clinical improvement.

Ultimately, randomised controlled trials assessing different management strategies based on quantitative bacterial load and qualitative microbiology results are needed and further investigation on inpatients (a more severe cohort) is required to assess the translatability of the results presented in this thesis.

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APPENDIX
Publications arising from this thesis:

BRONCHIECTASIS AN UPDATE ON CURRENT PHARMACOTHERAPY AND FUTURE PERSPECTIVES

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Abstract

Introduction: Bronchiectasis is a common condition and is likely to be underestimated, as bronchiectasis is now a recognized problem complicating other chronic lung diseases such as severe asthma, severe COPD and advanced pulmonary fibrosis. In more advanced bronchiectasis there is a vicious cycle of excess neutrophilic airways inflammation and chronic infection of the airways. This leads to the clinical syndrome including a chronic productive cough and recurrent chest infections.

Areas covered: This review provides an overview of the current pharmacotherapy options available and the potential future perspectives for treatment in adult patients with idiopathic or post infective bronchiectasis. A PUBMED search for all phase III and above trials on current therapies focusing on optimizing airway dilatation and treatments to break the vicious cycle of infection and inflammation were sought. These therapies include antibiotics, anti-inflammatory and mucoactive therapies alongside chest physiotherapy. Landmark phase II studies were also included.

Expert opinion: Current practice has predominantly been based on treatment advised from national guidelines that are mainly grade D expert opinion. Randomised controlled trials are greatly needed to improve practice of evidence based medicine.

Article Highlights

With the advent of CT, bronchiectasis is being increasingly recognized as an important clinical condition.
The treatments recommended depend on disease severity.
For milder disease, there should be a focus on education, regular chest physiotherapy, keeping up to date with influenza and pneumococcal vaccinations and receiving prompt antibiotics for infections.
For more severe patients, the above is essential with the additional consideration of a long-term antibiotic and/or a long term anti-inflammatory agent.
Large randomised controlled trials are needed.

Introduction

Bronchiectasis is a common chronic debilitating respiratory condition[1,2]. The gold standard for diagnosing bronchiectasis is using computed tomography of the chest as opposed to the bronchogram. Using modern imaging, the true prevalence is not known but we have over 750 patients with clinically significant bronchiectasis monitored in secondary care in Edinburgh, UK with a population around 480,000.

There are numerous causes of bronchiectasis with post-infection bronchiectasis being the commonest at 29-42% but no cause identified in 30-53% cases[3]. These can both lead to patients having daily cough, sputum production and recurrent chest infections[3,4].

The pathogenesis of bronchiectasis is poorly understood. Pulmonary pathology shows an excess of neutrophilic inflammation but despite this immune response, over two thirds of patients remain chronically infected with potential pathogenic microorganisms[5,6]. The driver for persistent neutrophilic airway inflammation in bronchiectasis is unknown, but infection is considered to play a major role.[6] Paradoxically, there is failure of clearance of bacteria from the airways and this leads to a 'vicious cycle' of infection and inflammation in the airways as first described by Cole and colleagues[4]. The neutrophils and bacterial products cause structural damage and perpetuate the vicious cycle. Therapies are needed to break this vicious cycle and indeed antibiotics and anti-inflammatory agents have the potential for this. The goals of treatment in this chronic condition are to reduce cough and sputum volume, reduce sputum purulence, reduce the number of chest infections and improve the health related quality of life.

This review will provide an overview on current pharmacotherapy and future perspectives in adult patients with clinically significant idiopathic and post infection bronchiectasis alone, as the management is the same. The review, however, does not cover the treatment of bronchiectasis due to active allergic bronchopulmonary aspergillosis, active sarcoid, cystic fibrosis and immunoglobulin deficiencies, which have their own disease specific treatments. We have included phase III and IV studies but where little evidence is available important phase II studies have also been included.

1. Antibiotics in exacerbations - Table 1

1.1 Role of oral antibiotics in exacerbations

When patients present with exacerbations, the British Thoracic Society (BTS) recommend sending sputum for bacteriological analysis but to start empirical antibiotics immediately[3]. The antibiotic recommended should be based on prior sputum microbiology but if no previous history is available, empirical amoxicillin (or clarithromycin in cases of penicillin allergy) should be started in addition to other supportive therapy. Antibiotics can be modified if patients fail to respond and this should be based on the pathogen isolated and the antimicrobial sensitivities. These recommendations are based predominantly on expert opinion (grade D) [3].

The evidence for oral antibiotic use in exacerbations is lacking, and even more so is the evidence for any particular agent. This may be due to the fact administration of antibiotics is so routine in treating chest infections. The length of optimal antibiotic treatment is still unknown and the British Thoracic Society recommend 14 days of antibiotics based on grade D evidence[3]. Below, we have presented some of the studies where oral antibiotics have been studied in bronchiectasis that could potentially guide management.

In an open labelled study, Hill *et al* investigated the response to oral antibiotics based on sputum purulence[7]. Antibiotics were given for exacerbations or in stable patients with intermittent mucopurulent or purulent sputum or in patients with persistent purulent sputum. All patients with mucoid (N=7) or mucopurulent (N=7) sputum had improved sputum purulence and/or clinical improvement when treated for an exacerbation with low dose amoxicillin 250mg t.d.s for 14 days, compared to 16% in those with persistently purulent sputum (N=19). Of those that had treatment failure, 58% then responded to high dose amoxicillin 3g b.d for 14 days. This study demonstrates that higher doses of prolonged antibiotics may be needed for patients with more severe bronchiectasis.

Chan *et al* investigated the use of ciprofloxacin versus amoxicillin in a randomised double blind trial with 42 patients[8]. Over a 7 day period they showed ciprofloxacin to have a better clinical response with reduced sputum volume, reduced purulence and higher bacterial eradication. Lam *et al* showed similar results with ofloxacin versus amoxicillin over 10 days with better microbiological clearance and clinical response with ofloxacin[9]. These cohorts of patients had *Pseudomonas aeruginosa* in 41% and up to 32% respectively in the baseline sputum microbiology and further confirms the need for targeted antibiotic use.

1.2 Is there an additional role for inhaled antibiotics in exacerbations?

Traditionally exacerbations are treated with oral or intravenous antibiotics. The study by Bilton and colleagues studied the effect of adding nebulised tobramycin to a 2 week course of oral ciprofloxacin for exacerbations of bronchiectasis due to *Pseudomonas aeruginosa*[10]. This double blind randomised controlled trial of 53 patients showed those treated concomitantly with inhaled tobramycin had a statistically significant reduction in bacterial density at day 7, which was maintained at day 14. There was however, no difference in clinical outcomes at day 14 (end of antibiotic treatment) and day 21 (one week after antibiotic treatment finished). Adverse events were common in both treatment arms with 22/26 in the tobramycin/ciprofloxacin arm and 26/27 in the placebo/ciprofloxacin arm reporting adverse events including wheeze, cough, dyspnoea, headache, fatigue and nausea. Only wheeze was reported at higher frequency in the tobramycin/ciprofloxacin arm with 24% of patients affected, compared to none in the placebo group. The wheeze did not seem to be predicted by the measurement of acute changes in FEV_1 measured 30mins post initial treatment and there was no significant difference in FEV_1 between the two groups at day 7 and day 14. In view that there was no added efficacy with nebulized tobramycin and there were more side effects, this study does

not support the routine addition of nebulized tobramycin for treatment of exacerbations due to *Pseudomonas aeruginosa*.

1.3 Role of intravenous antibiotics in exacerbations

There have only been limited studies in bronchiectasis exploring the efficacy of intravenous antibiotic therapy. As such the following recommendations by the BTS are based on grade D evidence only: Intravenous antibiotics should be considered if the patient is unwell and requires hospital admission, if resistant organisms are not responsive to oral antibiotics or if patients fail to respond to oral therapy (usually in patients infected with *Pseudomonas aeruginosa* or other enteric Gram-negative organisms)[3].

Tsang *et al* compared a 10 day course of oral levofloxacin with intravenous ceftazidime. They showed significant improvement in bacterial eradication rates, sputum volume, purulence, cough and dyspnoea scores in both groups[11].

As further evidence in bronchiectasis is lacking, we looked at trials conducted in large cohorts of patients that had bronchiectasis amongst other respiratory conditions. Brambilla *et al* studied lower respiratory tract infections (mainly patients with a pneumonia or exacerbation of chronic bronchitis and bronchiectasis), which showed similar clinical response rates to parenteral therapy with either co-amoxiclav or cefuroxime[12]. Both drugs had similar rates of clearance in sputum microbiology and adverse reaction rates. Mehta *et al* conducted a comparative study in patients with lower respiratory tract infections, of which 59% of the cohort had bronchiectasis[13]. They showed oral amoxicillin, oral co-amoxiclav and intravenous co-amoxiclav to have similar clinical and radiological improvement but co-amoxiclav to be superior in clearing bacteria from sputum (45% with co-amoxiclav vs. 8% with amoxicillin).

Overall there is a lack of trials exploring intravenous therapy in bronchiectasis. It seems pragmatic to follow the BTS guidelines[3]. Further studies are needed to explore which patients would benefit from intravenous as opposed to oral antibiotics, which intravenous agents to use and the optimal duration.

2. Stable state therapy

2.1 Role of airway bronchodilators

Airflow obstruction is frequently observed in bronchiectasis. Approximately 30% of patients with bronchiectasis have concomitant asthma or chronic obstructive pulmonary disease (COPD) and the role of β_2 agonist (both short and long acting) and anticholinergic therapy have been well described in these conditions. There are no randomised controlled trials that have investigated the effect of short and long acting β_2 agonists or anticholinergics in bronchiectasis[14-16].

Abu Hassan *et al* studied 24 bronchiectasis patients (asthmatic patients were excluded)[14]. Patients were given inhaled fenoterol 400micrograms by metered dose inhaler (MDI). Lung function was assessed at baseline and 30 minutes after inhalation. Patients were then given fenoterol 5mg via nebuliser and repeat lung function was repeated 30 minutes later. The study was repeated 24 hours later with

ipratropium bromide 40micrograms by MDI and with ipratropium bromide 500micrograms via nebuliser. 45.8% patients responded to one or both bronchodilators (>15% improvement in FEV_1), 12% responded to fenoterol alone and 12% to ipratropium alone[14].

In the stable state, regarding bronchodilator therapy, the BTS guidelines recommend assessment for airway obstruction and reversibility in all patients[3]. Inhaled therapy should be instituted when symptoms or lung function improves on β_2 agonist and/or anti-cholinergic therapy. Randomised controlled trials are needed to further assess the role of long term long acting β_2 agonists and anticholinergics in bronchiectasis.

2.2 Role of mucoactive therapies

Regular chest clearance is regarded as key for all patients with clinically significant bronchiectasis[3]. To aid this, there has been interest in treatments that improve mucociliary clearance and sputum expectoration. Hypertonic saline, mannitol, carbocysteine and DNase are the most studied agents but there are limited phase III trials in non-cystic fibrosis bronchiectasis patients.

Hypertonic saline and mannitol are hyperosmolar agents that improve airway hydration by inducing a liquid flux to the airway surface. This in turn helps to mobilise sputum.

In a small study by Kellett *et al* 24 patients were randomised to one of the 4 arms: 1) active cycle breathing technique (ACBT), 2) nebulised terbutaline and ACBT, 3) nebulised terbutaline, nebulised isotonic saline then ACBT and 4) nebulised terbutaline, nebulised hypertonic saline then ACBT[17]. They described improved ease of sputum expectoration, reduced sputum viscosity and a small improvement in lung function with the use of inhaled hypertonic saline (7%). More recently Kellett *et al* conducted a small randomised single blind trial of 28 patients where patients were given either nebulised 7% hypertonic saline or 0.9% isotonic saline for 3 months[18]. They reported those treated with hypertonic saline had a significant increase in FEV_1 by 15% from baseline compared to an increase of 1.8% with 0.9% isotonic saline ($p<0.01$). There was a significant ($p<0.05$) improvement in the quality of life of patients as assessed by the St George's Respiratory Questionnaire (SGRQ).

A randomised controlled trial by Nicolson *et al* investigated the effects of inhaling 6% hypertonic saline on quality of life and respiratory function[19]. 40 patients were randomised to receiving either 6% hypertonic saline (HTS) or 0.9% isotonic saline (IS) once daily for 12 months. They found both groups to demonstrate similar significant improvements in quality of life (as assessed by SGRQ), significant improvement in FEV_1 and FEF_{25-75} for both groups at 6 months but this was not sustained at 12 months for FEV_1. There was a significant reduction in sputum colonisation from 60% in the IS group and 55% of HTS group at baseline to 15% in both groups at 12 months. There was no difference between the groups in any of the endpoints including exacerbation frequency. There were 3 adverse events in the HTS group (chest tightness due to underlying exacerbation, transient hypertension not requiring treatment and fast atrial fibrillation) with only 1 patient electing to cease treatment. There were no adverse events in the IS group. Larger randomized

controlled studies are needed before the routine use of hypertonic saline can be advocated.

A Cochrane review on the use of mannitol in bronchiectasis identified only 2 small randomised studies and several non-randomised studies[20]. Daviskas *et al* reported doubling of tracheobronchial clearance 75 minutes after inhalation of 400mg mannitol when compared with coughing and inspiratory manoeuvres alone[21]. Lung function did not significantly change and mannitol was well tolerated despite the induction of cough[22-24]. An optimum dose of 480mg of mannitol induced most sputum clearance but must be administered twice daily in order to achieve sustained effects[23]. Quality of life was improved by a reduction of 12.4units in the SGRQ (four units being the minimal clinically important difference) [24].

More recently a phase III randomised study was conducted by Bilton *et al* to assess the efficacy and safety of dry powdered mannitol[25]. Patients were randomised to inhale 320mg mannitol (n=231) or placebo (n=112) twice daily for 12 weeks. The mannitol treated group reported improved sputum expectoration and in a subgroup study (n=82) mannitol subjects showed less small airway mucus plugging on HRCT at 12 weeks compared to placebo (p=0.048). There was no statistical difference between the groups (p = 0.304) for total SGRQ score - Mannitol -3.4 points (95% CI: -4.81 to -1.94) versus placebo -2.1 points (95% CI: -4.12 to -0.09)). Compliance rates were high and mannitol was well tolerated with similar adverse events to placebo. A larger controlled study is now required to investigate the long-term effects of mannitol on pulmonary exacerbations and antibiotic use.

Mucolytics such as DNase, carbocysteine and bromhexine have also been investigated to enhance mucociliary clearance by changing the physiochemical properties of sputum. This reduction in sputum viscosity should allow easier mobilisation. A Cochrane review identified only 3 studies (described below) assessing mucolytics in bronchiectasis patients[26]. Mucolytics remain a promising prospect for treatment but randomised controlled data is lacking to support any recommendations for regular use.

The first study by Olivieri *et al*, assessed bromhexine 30mg t.d.s versus placebo[27]. They noted an improvement in 'difficulty in expectoration', an increase in sputum production and a significant reduction in cough. There was no change in lung function. Wills *et al* conducted a double blind randomised control trial studying human recombinant DNase (rhDNase) 2.5mg twice daily, 2.5mg once daily, placebo twice daily or placebo once daily in 21 patients with bronchiectasis[28]. They failed to show any improvement in lung function, dyspnoea or quality of life at the higher dose but did report a significant improvement in dyspnoea with low dose rhDNase. Influenza-like symptoms were reported in 4 subjects randomised to high dose rhDNase. O'Donnell *et al* conducted a large double blind randomised control trial investigating rhDNase versus placebo twice daily for 24 weeks in 349 patients[29]. The authors reported a significant increase in protocol derived and non-protocol derived exacerbation rates when compared with placebo. They also reported a significant reduction in FEV_1 compared with placebo (FEV_1 decline -3.7% change

vs -1.7% in placebo group) and as a consequence of this trial DNase is not recommended in the treatment of bronchiectasis[3].

2.3 Role of corticosteroids - Table 2

Oral steroids have long since been used in difficult to treat asthma by limiting local and systemic inflammation. There are currently no randomised controlled studies that have investigated the role of oral corticosteroids in bronchiectasis.

A Cochrane review identified limited studies for the use of inhaled corticosteroids in bronchiectasis[30]. Tsang *et al* demonstrated in a double-blind placebo controlled trial that 4 weeks of inhaled corticosteroids (ICS) reduce sputum inflammatory markers Interleukin (IL)-1, IL-8, leukotriene (LT)B_4 and total neutrophil count[31]. There was no reduction in exacerbation frequency, sputum volume, bacterial density or lung function. A further study by the same group randomized 86 patients to inhaled fluticasone or placebo for 12 months and showed a statistically significant reduction (20%) in sputum volume in those receiving inhaled fluticasone and in a sub analysis there appeared to be a particular benefit to patients colonised with *Pseudomonas aeruginosa*[32] .

Elborn *et al* conducted a small placebo controlled double blind 6 week crossover study examining inhaled beclometasone diproprionate[33]. They showed a significant reduction (18%) in sputum volume and small improvements in FEV_1 and peak expiratory flow rates. The clinical significance of this small improvement in FEV_1 and peak flow rates is unknown. Martinez *et al* showed that the use of high dose ICS over 6 months improved quality of life in bronchiectatic patients[34].

Combination therapy with inhaled corticosteroid and long acting β_2 agonists may also have a role in bronchiectasis. Martinez-Garcia *et al* showed combination therapy with budesonide-formoterol inhalers was more efficacious than high dose budesonide alone. The double blind randomised study reported statistically significant improvement in symptoms and health related quality of life in the combination group. In addition, there were fewer side effects with combination therapy, especially those associated with inhaled steroids[35].

The above studies suggest inhaled corticosteroids reduce sputum volume and improve health related quality of life. The studies however have not proven whether long term treatment has any impact on exacerbations. In addition, there have been concerns about the long-term use of inhaled corticosteroids in patients with COPD with regards to pneumonia risk[36]. Before advocating long term treatments, the benefits must outweigh the potential risks. At this current time, the routine use cannot be recommended[3]. Large randomized controlled studies are needed.

2.4 Other potential anti-inflammatory treatments

2.4.1 NSAIDS: There are no randomised controlled trials assessing the efficacy of oral NSAIDS in bronchiectasis. A Cochrane review identified only 1 small randomised double blinded controlled study comparing inhaled indomethacin 2.4micrograms, t.d.s for 14 days in 25 patients with chronic lung disease (8 bronchiectasis, 12 chronic bronchitis and 5 diffuse panbronchiolitis) with

placebo[37,38]. Results showed an improvement in the amount of sputum production and dyspnoea as assessed by the Borg's score but no change in lung function. Another small study of 9 stable bronchiectasis patients included the treatment of 4 weeks of oral indomethacin therapy (25mg, t.d.s). Analysis showed marked reduction in peripheral blood neutrophil function with reduced chemotaxis and fibronectin degradation but no change in airway bacterial load or sputum chemotaxis[39]. Larger randomised controlled trials are needed to assess the use of NSAIDs as potential anti-inflammatory agents before they can be considered for routine treatment.

2.4.2 Statins: Statins are widely used in cardiovascular disease and have been shown to be associated with a 41% reduction in 30-day mortality in patients hospitalized with seasonal influenza[40]. One of the pleotropic effects of statins is their anti-inflammatory property, which could have a role as a novel, non-antibiotic treatment in bronchiectasis. There are no randomised controlled trials that have been conducted evaluating their routine use in bronchiectasis but this could be a potential anti-inflammatory therapy for the future.

2.4.3 Others: Because of the excess neutrophil burden in bronchiectasis, future studies are needed to assess novel therapies such as neutrophil elastase (NE) inhibitors and tumour necrosis factor alpha (TNFα) inhibitors but to date there has only been one small study on neutrophil elastase inhibitors and none in TNFα inhibitors.

Stockley *et al* randomised 40 patients to receiving NE inhibitor AZD9668 60mg, b.d. (n=22) or placebo (n=16) for 4 weeks[41]. They reported a significant improvement in FEV_1 of 100mls, slow vital capacity, plasma interleukin 8 and post waking sputum interleukin 6. There was no significant improvement in quality of life, sputum weight or other lung function tests in the treatment group. AZD9668 seemed to be well tolerated with fewer adverse events reported than in the placebo arm. The most common complaint in the treatment arm was of headache 7/22 compared with 2/16 in the placebo arm. These results are encouraging but larger studies of longer duration are needed.

2.5 Role of antibiotics in the stable state

2.5.1 Role of long-term oral antibiotics

Studies have shown that recurrent exacerbations lead to a poorer quality of life in bronchiectasis. One of the key aims of management of bronchiectasis is improving quality of life in these patients. Several studies have investigated long term antibiotic therapy in stable patients with the aim of reducing exacerbation frequency and thereby improving quality of life.

The key studies investigating long term oral antibiotics are outlined in Table 3. In the infancy of this condition, randomised controlled trials assessed the impact of long-term tetracyclines and penicillins. The Medical Research Council[42] conducted a randomised controlled trial of 122 patients over the course of a year. Patients were randomised to 2g penicillin, 2g tetracycline or 2g lactulose (placebo). Findings reported tetracyclines to be more effective than penicillin in reducing symptoms and

sputum volume with less days confined to bed and less days off work. A further randomised trial by Sobel and colleagues assessed for adverse reactions with long-term antibiotics and found 27% of patients treated with tetracyclines developed side effects and predominantly diarrhoea[43].

Cherniack and colleagues assessed the long-term impact of 2g oral tetracycline/day vs. 1g oral penicillin/day vs. 2g oral oleandomycin/penicillin combination vs. placebo over a period of at least 3 months (extending up to 22months) on exacerbation frequency in patients with chronic bronchitis and bronchiectasis[44]. They reported fewer lower respiratory tract illnesses with tetracyclines compared to either placebo or penicillin alone and that penicillin alone was not more effective than placebo. The combination treatment was also better at reducing exacerbation frequency than placebo. A similar study by Dowling and colleagues assessing the effect of the same 4 arms on sputum microbiology over a period of at least 3 months (extending up to 31 months) showed that whilst tetracyclines reduced the presence of *H. influenzae*, *S. pneumoniae* and *S. aureus* there was an increase in *Pseudomona*s species[45].

Currie *et al* showed a reduction in exacerbation severity, but not frequency, with high dose amoxicillin (3g, b.d) administered over a 32 week period when compared with placebo[46]. They also reported a reduction in sputum purulence, volume and less time confined to bed or off work.

Whilst these studies are old, we thought it prudent to include them here, as there has been increasing use of simpler antibiotics such as amoxicillin and doxycycline in clinical practice. In addition, there is a lack of randomised controlled trial data on non-macrolide antibiotics in stable bronchiectasis.

2.5.2 Role of macrolide therapy - Table 4

In addition to antibacterial properties, macrolides are thought to have immunomodulatory and anti-inflammatory properties. Over recent years, several studies have been done assessing the role of long term macrolides in stable bronchiectasis. Here we outline the key trials and summarise them in Table 4.

Tsang *et al* performed a randomised double blind placebo controlled study showing an improvement in lung function and sputum volume reduction in patients treated with 8 weeks of 500mg b.d erythromycin[47]. It did not show a difference in microbial load or inflammatory markers but the majority of patients were colonised with *Pseudomonas aeruginosa*.

Wong *et al* confirmed these findings in the Effectiveness of Macrolides in patients with Bronchiectasis using Azithromycin to control Exacerbations (EMBRACE) trial[48]. This was a randomised double-blind placebo controlled trial of 141 patients randomised to either taking azithromycin 500mg three times a week or placebo for 6 months. They showed a significant reduction in the rate of event-based exacerbation from 1.57 in the placebo group to 0.59 with the azithromycin group. There was no statistically significant difference in lung function or quality of life. Macrolide resistance testing was not routinely undertaken in this study but 4% in the

azithromycin group had macrolide resistant *Streptococcus pneumoniae* at 6 months.

More recently the Bronchiectasis and long term Azithromycin Treatment (BAT) study in 83 bronchiectasis patients with 3 or more exacerbations in the past year were randomised to either 250mg azithromycin once daily or placebo for 1 year[49]. Results showed a significant decrease in exacerbation frequency in the azithromycin group with a longer time to first exacerbation during treatment. FEV_1 and FVC improved with azithromycin, as did quality of life as assessed by SGRQ. Sputum microbiology was similar at baseline and at 1 year but macrolide resistance was recorded in 88% of the isolates tested compared with 26% in the placebo group. Adverse reactions were reported, mainly gastrointestinal symptoms, but these were not severe enough to discontinue treatment.

The Bronchiectasis and Low dose Erythromycin Study (BLESS) investigated the effect on exacerbations rates and resistance rates post 1-year therapy with low dose erythromycin[50]. Patients were randomised to low dose erythromycin or placebo for 48 weeks. Results showed a significant reduction in exacerbation frequency, reduced FEV_1 decline but an increase in macrolide resistance. Erythromycin was well tolerated without any evidence of significant adverse effects. In the literature, there is evidence of increased risk of cardiac death due to prolonged QTc and arrthymias with the use of macrolides[51]. However, no difference in QTc values or arrthymias was found at this low dose, in the BLESS trial. Erythromycin increased the proportion of macrolide resistant oropharyngeal streptococci.
The potential long term use of macrolides is discussed in the expert opinion section.

2.6.3 Role of long term inhaled antibiotics

Nebulised antibiotic therapies target the airways directly and should thereby reduce systemic side effects. Several studies have investigated the role of long term nebulised antibiotics in the stable state and are summarized below. These key studies are also listed in Table 5.

The initial proof of concept study by Lin *et al* showed the acute efficacy of inhaled gentamicin over 3 days and led the way for long term antibiotics studies in the stable state[52]. They assessed the effect of inhaled gentamicin on airway neutrophil activity and mucus hypersecretion in 28 stable patients with bronchiectasis. Patients were randomised to inhaled gentamicin 40mg b.d or 0.45% saline bd. No baseline microbiology was recorded. In the gentamicin arm, they reported reduced sputum myeloperoxidase, reduced bacterial load and sputum volume. In addition, they reported improved breathlessness, exercise tolerance and peak expiratory flow rates.

Barker *et al* administered nebulised tobramycin 300mg b.d versus placebo to patients colonised with *Pseudomonas aeruginosa*[53]. There was a significant reduction in bacterial density at week 4 and eradication in 35% of patients at week 6 (2 weeks after completing treatment). However, 70% of patients in the tobramycin arm reported respiratory side effects compared to 51% of placebo patients. Of the adverse effects reported in the tobramycin arm, 12 out of the 15 that reported increased cough, 3 out of the 6 that reported wheeze, 3 out of the 7 that reported chest pain, 3 out of the 12 that reported breathlessness adverse effects were thought to be due to the tobramycin itself.

Recently, Wilson and colleagues studied the effects of inhaled ciprofloxacin dry powder for inhalation (DPI) 32.5mg twice daily versus placebo for 28 days in a randomised double-blind study[54]. Their primary end point of bacterial load showed a reduction in sputum bacterial load in treatment arm patients at day 28 but that bacterial counts increased thereafter in the 56 day follow up. The treatment was well tolerated as only 3 out of the 60 recruited patients in the treatment arm reported bronchospasm, 0 out of 60 reported cough and 1 out of 60 reported haemoptysis.

To investigate nebulised therapy as a longer-term therapy Drobnic *et al* administered nebulised tobramycin 300mg b.d in a randomised trial over 6 months[55]. They found a significant reduction in number and length of hospital admissions, reduced bacterial density and no increase in bacterial resistance. No difference in exacerbation frequency, lung function or quality of life (QofL) was detected and bronchospasm was reported in 10% of patients.

Serisier and colleagues conducted a multicentre phase II double-blind placebo-controlled randomised trial assessing the effects of regular intermittent treatment with dual release ciprofloxacin for inhalation (DRCFI) containing liposomal ciprofloxacin, in a subset of bronchiectasis patients infected with *Pseudomonas aeruginosa*[56]. Patients received 3 treatment cycles of 28 days on and then 28 days off treatment (liposomal ciprofloxacin versus placebo). They reported a significant reduction in bacterial count on day 28 when compared with placebo and also reported bacterial counts trended back up to baseline during treatment off periods but only 1/22 patient in the placebo group and 5/20 patients in the treatment group finished the 3 cycle trial. There was a significant delayed time to first exacerbation but only in a per protocol analysis. Systemic adverse events were similar in both arms but pulmonary adverse events were lower in the DRCFI group.

Similarly, Orriols *et al* showed treatment with nebulised therapy (ceftazidime and tobramycin) over 1 year was effective in reducing hospital admissions and length of hospital stay with no increase in antibiotic resistance[57]. Murray *et al* conducted a randomised trial investigating the use of long term nebulised gentamicin 80mg twice daily vs. 0.9% saline in bronchiectatic patients irrespective of baseline microbiology[58]. The researchers were blinded but patients were not. Main study findings were a reduction in bacterial density, reduced sputum purulence, greater exercise capacity and fewer exacerbations. Time to first exacerbation was also increased and there was an improvement in cough and quality of life as assessed by Leicester Cough Questionnaire and St George's Respiratory Questionnaire respectively. 30.8% eradication in those with *Pseudomonas* infection and 92.8% eradication in those infected with other pathogens was reported. All markers returned to baseline 3 months post cessation of treatment suggesting the need for continuous therapy or less than 3 months off therapy for sustained efficacy.

The potential long term use of inhaled antibiotics is discussed in the expert opinion section.

3. Conclusions

There is a distinct lack of randomised controlled trials in this common chronic condition. Considering this, many of the recommended treatments are based on expert guidance. In clinical practice the mainstay of treatment is airways chest clearance using chest physiotherapy, optimizing the airways with short and long acting bronchodilators +/- inhaled corticosteroid, preventing exacerbations (with vaccinations and consideration of a long-term antibiotic) and prompt treatment of exacerbations. Future studies are needed to improve the evidence of the role of existing and new therapies.

4. Expert opinion and future perspectives

Bronchiectasis was first described in 1819 and despite this the field has been hampered by the lack of randomised controlled trials. In a PUBMED search October 2013 there were 151 randomised controlled trials in bronchiectasis compared with 5,754 in lung cancer. In light of this, the recommendations regarding treatment are largely based on expert opinion.

There is a spectrum of severity in bronchiectasis. In mild bronchiectasis patients can be asymptomatic, are not normally chronically colonised when clinically stable, have no pathogens in their sputum, have infrequent chest infections (fewer than three annually) and mild radiological bronchiectasis. In severe bronchiectasis patients are chronically colonised when clinically stable usually with *Pseudomonas aeruginosa*, other enteric gram negative pathogens or Methicillin Resistant *Staphylococcus aureus*, have frequent chest infections (three or more annually) and advanced radiological changes with varicose and cystic bronchiectasis[3].

This review identifies the current and relevant evidence to guide treatment for idiopathic and post-infection bronchiectasis. The treatment goals for clinically significant disease include the reduction of cough, sputum volume, sputum purulence, number of chest infections and to improve quality of life. The treatment differs depending on the severity of disease. The emphasis for milder patients concentrates on education, regular chest physiotherapy, keeping up to date with influenza and pneumococcal vaccinations and receiving prompt antibiotics for infections. For more severe patients, the above is essential with the additional consideration of a long-term antibiotic and/or a long term anti-inflammatory agent.

Patients have permanently damaged airways and are prone to having a cough, sputum production and recurrent chest infections. There is reasonable consensus that antibiotics are required for exacerbations when patients feel a deterioration associated with increased sputum volume and purulence. The commonest pathogenic organisms are *Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Moraxella Catarrhalis and Streptococcus pneumoniae*[5,6]. The concept of targeting antibiotics based on the pathogen isolated from sputum bacterial culture is sound and will ensure that appropriate antibiotics are given to cover the suspected pathogen. It will also allow simplifying antibiotic choice rather than using broader spectrum antibiotics for all exacerbations. The British Thoracic Society Guidelines recommend 14 days of antibiotics for all exacerbations[3]. This is based on the hypothesis that patients with damaged airways and increased bacterial

load require a higher dose and prolonged course of antibiotics to clear an infection. Whilst this hypothesis in general is sound, there is a wide spectrum of severity of bronchiectasis. Whilst 14 days is pragmatic for more severe exacerbations, a shorter course such as 7 days, will likely be effective for milder cases of bronchiectasis. Randomised controlled trials are needed to assess the optimum duration of antibiotics in mild, moderate and severe bronchiectasis.

The current mode of delivery seems pragmatic- for exacerbations to start off with oral antibiotics and to consider intravenous antibiotics if patients are ill and require hospital admission, have pathogens resistant to oral antibiotics or have failed to respond to an appropriate course of oral antibiotics. The addition of inhaled antibiotics to oral antibiotics in the short term did not improve outcomes and led to more adverse effects[10] and currently is therefore not routinely recommended. Future studies addressing whether inhaled antibiotics are additive to intravenous antibiotics needs further study and would be of potential benefit in patients with more severe bronchiectasis with drug resistant pathogens.

There is some controversy whether to prescribe antibiotics if patients present with a viral exacerbation. Patients will often describe starting with a viral infection, which subsequently leads to a secondary bacterial infection. Whilst no studies have been conducted in bronchiectasis, a recent study performed in both COPD subjects and healthy volunteers showed an increase in bacterial burden in the COPD cohort only (16% rise in proteobacterial sequences, most notably in *Haemophilus influenzae* from baseline) after inoculation with rhinovirus[59]. This may be of relevance in bronchiectasis also and studies are needed to investigate if viruses alter the respiratory microbiome and whether early intervention with antibiotics can reduce these secondary bacterial infections or not. In clinical practice, we tend to advise patients to keep rescue antibiotics at home and if there is increased sputum volume and purulence to start antibiotic therapy at that stage.

Reducing respiratory tract infections is important to reduce the burden of disease. Although there is a lack of randomised controlled trials all patients should receive the annual influenza vaccination and the pneumococcal vaccination, which may be repeated every 5 years[3]. Another clear recommendation is to advise patients to avoid contact, if possible, with people known to have the cold or flu.

The first line therapy for bronchiectasis is to educate the patient about their condition. They should know when and how to perform chest physiotherapy and when to seek medical help for an infection. There is a national standard that has been written for bronchiectasis and this recommends that all patients should see a dedicated respiratory physiotherapist and to be taught airway clearance techniques[60]. The standard technique used in current day practice is the acute cycle breathing technique (ACBT). There are many other techniques available and another commonly used technique is autogenic and postural drainage. Sometimes positive expiratory airway pressure devices such as the acapella or flutter valve are used to aid sputum clearance. Despite the lack of randomised controlled trials in bronchiectasis, there is clear scientific rationale to clear the purulent secretions from the airways, which will improve symptoms but may also reduce the incidence of

respiratory tract infections. In mild disease, this may only need to be done during respiratory tract infections but in patients with severe disease, patients should do this at least twice daily.

There has been increasing interest in airway adjuncts to promote airways clearance. Large multi-centred randomised controlled trials are needed but there is encouraging data that suggests both hypertonic saline and mannitol may promote airway clearance[17,18,19,25]. We do however need studies to show if these treatments can ultimately reduce exacerbations. The study investigating DNase by O'Donnell *et al*, is an important landmark study showing that DNase was harmful in bronchiectasis and caused increased exacerbations and an accelerated decline in lung function[29]. This was a key study showing that we cannot just adopt treatments that work for cystic fibrosis in patients with bronchiectasis.

In addition to chest clearance, patients with bronchiectasis with airflow limitation and breathlessness should have a trial of short and long acting bronchodilators. Further studies are needed to address which patients may benefit from inhaled corticosteroids, as both reducing sputum volume and improving health status are important[31-35]. The studies however have not proven whether long term treatment has any impact on exacerbations. In addition, there have been concerns about the long-term use of inhaled corticosteroids in patients with COPD with regards to pneumonia risk[36]. Before advocating long term treatments, the benefits must outweigh the potential risks. At this current time, the routine use cannot be recommended except if there was co-existent asthma or significant COPD[3]. Large randomized controlled studies are needed and in addition, further research is needed to address whether inhaled corticosteroids are additive to long term antibiotic and mucoactive therapy.

There has been a growing interest in newer therapies for bronchiectasis to break the 'vicious cycle'. These include anti-inflammatory and anti-infective therapies. It is likely such treatments are not needed for patients with mild bronchiectasis who have few symptoms when clinically stable and have few chest infections. The largest anti-inflammatory therapies studied to date are the macrolides. There is however debate whether macrolides are anti-infective, anti-inflammatory or both. There are three randomised controlled studies using macrolide therapy for 6 months up to one year. All three studies showed in comparison to placebo, macrolide therapy reduced exacerbations[48-50]. It is clear that it was not dependent on the macrolide chosen or the frequency of delivery. Reducing exacerbations is key to improving the morbidity of bronchiectasis and these studies support the long-term use of macrolides. There are however concerns with macrolides concerning their toxicity in a middle age and elderly cohort. The concerns are the risk of non-tuberculous mycobacteria, cardiovascular mortality and pneumococcal resistance to macrolides[61,62]. In bronchiectasis, macrolide resistance in this group would make the treatment of non-tuberculous mycobacteria very difficult to treat. This is of major concern because non-tuberculous mycobacteria are isolated in 5-10% of cases in Europe but 30-40% cases in the US[61,62]. Recent reviews have shown that macrolide use can increase cardiovascular mortality, which would be concern to a middle and elderly aged cohort[51,63]. It has been shown in these studies that there is a significant increase in

pneumococcal resistance to macrolides[49,50]. It is not known however whether this has any clinical significance. In the studies to date up to one year, these have not been of any clinical significance.

There are many potential new anti-inflammatory treatments in bronchiectasis. The aetiology of bronchiectasis is poorly understood and in over 50% there is no identified aetiology[3]. There is growing interest in assessing whether channelopathies including CFTR are implicated in bronchiectasis. If identified, this offers a potential novel new treatment target. There are a whole host of potential new anti-inflammatory treatments but in our opinion the following targets would be an excellent start. These include airway anti-inflammatories such as inhaled anti-elastase therapy, alpha-1-antitrypsin or secretory leukoprotease inhibitor, targets to reduce neutrophil recruitment such as monoclonal antibodies against interleukin 8, leukotriene B4 and C5a, pro-resolution treatments such as lipoxins and resolvins, and systemic anti-inflammatory therapies such as statins or anti-tumour necrosis factor alpha blockers.

Long term oral antibiotics have been proposed as a treatment for patients with three or more exacerbations annually despite other therapy[3]. These are suitable particularly in patients colonised with potential pathogenic organisms, for example *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*, which have multiple suitable oral antibiotic preparations. In patients with recurrent exacerbations a long term oral antibiotic may reduce the bacterial burden and improve outcomes[42,44,46]. The major limiting factor is that such long-term antibiotics have side effects and predominantly diarrhoea[43,45]. We would recommend the antibiotic chosen be based on the pathogen and sensitivity testing and continued long term. Further studies are needed to address whether such treatments can impact on bacterial resistance.

A recent growing area has been in the field of long term inhaled antibiotics. The studies to date show exciting preliminary data that long term inhaled antibiotics can reduce the bacterial burden and improve clinical outcomes[55-58]. We need however more studies to confirm that long term inhaled antibiotics can reduce exacerbations. There has been a longstanding debate whether these treatments should be one month on and off or continuous[56,58]. The theory for the on-off regimen was to reduce the development of microbial resistance. The long term antibiotic studies to date have not shown that continuous antibiotics lead to bacterial resistance affecting patient care[55,57,58]. In our opinion continuous treatment allows the optimum therapy to reduce the bacterial load and ultimately reduce exacerbations. There will be a wide spectrum in the future of potential inhaled antibiotics and the agent chosen should be based on the pathogen and sensitivity patterns. It is likely that inhaled therapy will be predominantly used in patients colonised with more complex pathogens such as *Pseudomonas aeruginosa* where there are limited or no long term suitable oral therapies. The limiting factors to date using these therapies are they have significant cost to their use and are all currently unlicensed and therefore their use is confined to registered clinical trials. In addition, such therapies can cause bronchospasm and breathlessness and around 10% of patients will have to discontinue therapy despite the addition of inhaled or nebulised bronchodilator therapy[53,55,58].

An alternative approach in patients with very severe bronchiectasis is the consideration for regular planned eight weekly courses of intravenous antibiotic therapy. Our open labelled study showed that this treatment improved patients' symptoms with reduced antibiotic burden, cough and improved health status and reduction of systemic inflammation[64]. Randomised controlled trials are however needed to explore this further.

There is an international drive to license both anti-infective and anti-inflammatory therapies for this chronic disabling condition using traditional medicines. There will likely be novel new therapies in the future attempting to modify the inflammatory or host response. The future will evaluate whether anti-inflammatory therapies are additive to antibiotic therapies as would be expected in the vicious cycle seen in bronchiectasis. The next 10-20 years will see a large expansion in this field, which will improve the evidence base for this chronic disease.

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Author	Study	N	Antibiotic	Dose & Duration	Microbiology	Results	Our Interpretation
Hill *et al*, 1986[7]	Open label	33	Oral Amoxicillin	250mg t.d.s 14 day	Initial responders: 47% no pathogen 35% *H. influenzae* 12% *S. pneumoniae* 6% *P. aeruginosa*	7/7 mucoid to clear and time to next exacerbation 6.5 months 7/7 mucopurulent to clear and time to next exacerbation 9 days 3/19 purulent to clear and time to next exacerbation 4 days	Patients with purulent sputum need higher dosages of prolonged antibiotics.
			Oral Amoxicillin	3g b.d 14 days, if failed to respond to above	Those given 2nd line: 31% no pathogen 50% *H. influenzae*	7/12 (4 patients not entered) purulent to clear and time to next exacerbation 14 days	
			Nebulised Amoxicillin	500mg b.d 4 months, if failed to respond to above	13% *P. aeruginosa* 6% *S. aureus*	2/3 (2 patients not entered) purulent to clear with no relapse at 11 months	
Chan *et al*, 1996[8]	Randomised	42	Ciprofloxacin Versus. Amoxicillin	500mg b.d 7 days 1g t.d.s 7 days	41% *P. aeruginosa*	100% bacterial clearance 95% improved sputum purulence 25% bacterial clearance 55% improved sputum purulence	Targeted antibiotics improve bacterial clearance.
Lam *et al*, 1989[9]	Randomised	41	Ofloxacin Versus. Amoxicillin	200mg t.d.s 10days 1g t.d.s 10days	32% *P. aeruginosa* or *Klebsiella*	14/20 'excellent' improvement in sputum purulence 1/14 'failure' in improvement 94% bacterial eradication 8/21 'excellent' improvement 8/21 'poor' improvement 45% bacterial eradication	Targeted antibiotics improve bacterial clearance.
Bilton *et al*, 2006[10]	Randomised	53	Ciprofloxacin + inhaled tobramycin Ciprofloxacin + placebo (quinine sulphate)	750mg oral ciprofloxacin + 300mg/5ml of Tobramycin inhalation solution b.d for 14 days 750mg oral ciprofloxacin + 1.25mg/5ml of quinine sulphate b.d for 14days	100% *P. aeruginosa*	No statistically significant difference in clinical outcomes at days 14 or 21 despite greater microbiological response with the addition of inhaled tobramycin. Increased wheeze with tobramycin 50% vs. 15% (placebo arm).	The addition of inhaled tobramycin to oral ciprofloxacin confirmed no additional clinical benefit but increased symptoms related to inhaled tobramycin.
Tsang *et al*, 1999[11]	Randomised	35	Levofloxacin Versus. IV Ceftazidime	300mg b.d 10 days 1g t.d.s 10 days	29% *P. aeruginosa* 41% no pathogen	57% bacterial clearance 69% bacterial clearance Significant improvement in eradication rates, sputum volume, purulence, cough and dyspnoea scores in both groups. No significant difference between groups.	No difference in bacterial clearance comparing oral with intravenous anti-pseudomonal antibiotics.
Brambilla *et al*, 1992[12]	Comparison	512	IV and then oral Cefuroxime axetil IV and then oral Amoxicillin plus clavulanic acid	750mg, t.d.s, iv (48-72hr) then 500mg, b.d, oral (5days) 1.2g, t.d.s, (48-72hr) then 625mg, t.d.s, oral (5days)	Not available	87.1% cured or improved 72.8% bacterial clearance 85.9% cured or improved 70% bacterial clearance	Similar clinical and microbiological efficacy comparing cephalosporins and penicillin with clavulanic acid therapy.

Mehta *et al*, 1991[13]	Comparison	85	Amoxicillin Amoxicillin plus clavulanic acid Amoxicillin plus clavulanic acid	500mg, t.d.s, oral 7-10days 750mg, t.d.s, oral 7-10days 1.2g, t.d.s, IV 3 days then oral as above	*S. pneumoniae* (n=12) *Klebsiella* (n=41) *P. aeruginosa* (n=21) *E. coli* (n=9) *H. influenzae* (n=7) *S. aureus* (n=6)	66% clinical improvement 47% radiological improvement 60% clinical improvement 53% radiological improvement 56% clinical improvement 44% radiological improvement 45% bacterial clearance with amoxicillin plus clavulanic acid compared with 8% bacterial clearance with amoxicillin.	Targeted antibiotics improve bacterial clearance.
Table 1. Antibiotics in bronchiectasis exacerbations							

Author	Study	N	Corticosteroid	Dose & Duration	Results	Our Interpretation
Tsang *et al*, 1998[31]	Randomised	24	Fluticasone Versus Placebo	500micrograms, b.d, 4 weeks	The fluticasone group had a significant decrease in sputum leukocyte count, IL-1β, IL8 and LTB_4. The fluticasone group had one and the placebo group three exacerbations (not statistically significant). No significant change in spirometry or sputum volume. No adverse reactions were reported in either group.	This proof of concept study showed inhaled corticosteroids over 4 weeks reduced sputum neutrophilic airways inflammation.
Tsang *et al*, 2005[32]	Randomised	86	Fluticasone Versus Placebo	500micrograms, b.d, 1 year	The fluticasone group had a significant improvement in 24hr sputum volume (OR 2.5, 95% CI 1.1 to 6.0, p = 0.03) but not exacerbation frequency, sputum purulence or lung function. Patients colonised with *Pseudomonas* had significant improvements in sputum volume (OR 13.5, 95% CI 1.8 to 100.2, p = 0.03) and exacerbation frequency (OR 13.3, 95% CI 1.8 to 100.2, p = 0.01).	This study showed long term inhaled corticosteroid over 1 year reduced sputum volume and in a subanalyis showing benefit particularly in patients with *Pseudomonas aeruginosa*. Further confirmatory studies are needed.
Elborn *et al*, 1992[33]	Randomised	20	Beclometasone dipropionate Versus Placebo	750micrograms, b.d, 6 weeks	The beclometasone group had a significant (18%) reduction in sputum volume (p<0.003).	This study showed a reduction in sputum volume over 6 weeks.
Martinez *et al*, 2006[34]	Randomised	93	High dose Fluticasone Versus Low dose fluticasone Versus No treatment	500micrograms, b.d, 6 months 250micrograms, b.d, 6 months No treatment 6 months	Only high dose inhaled steroid showed a significant improvement in dyspnoea, sputum production, days without cough, short acting β_2 agonist use and quality of life. The SGRQ improved from (45.4 [14.2] vs. 40.5 [13.9]; P = 0.01). There were no changes in pulmonary function, sputum microbiology or number or severity of exacerbations. Increase in adverse effects with high dose ICS, more commonly of dry mouth, local irritation and dysphonia. Significant reduction in sputum volume with the lower dose steroid but not maintained at 6months.	This study showed only clinical benefit with dose inhaled corticosteroid.
Martinez *et al*, 2012[35]	Randomised	40	Formoterol-budesonide Versus	18/640micrograms, 3 months	In the combination treatment arm there was a significant improvement in dyspnoea, increase in cough free days, a reduction in number of rescue β_2 agonist inhalations and improvement in QofL. The SGRQ in	The combination therapy improved symptoms and had less side effects from high dose inhaled

			Budesonide	1600micrograms, 3 months	the combination treatment improved by 5.3 units; *P*= 0.006). No difference in lung function or sputum microbiology. More exacerbations in the high dose budesonide group but not statistically significant from the combination arm. The combination treatment had less inhaled corticosteroid side effects such as pharyngeal irritation (5% vs. 35%), dysphonia (0% vs. 25%) and mouth dryness (5 vs. 35%).	corticosteroids.
Table 2. Inhaled corticosteroids in bronchiectasis						

Author	Study	N	Antibiotic	Dose & Duration	Microbiology	Results	Our Interpretation
MRC, 1957[42]	Randomised	122	Penicillin Tetracycline Lactulose	2g 1yr, 2x/week 2g 1yr, 2x/week 2g 1yr, 2x/week	None recorded	Sputum purulence improved by 33% 26% reduction in volume Sputum purulence improved by 50% 36% reduction in volume Sputum purulence improved by 29% 24% reduction in volume	First long term randomised controlled trial showing long term tetracycline improved clinical outcome.
Sobel *et al*, 1962[43]	Randomised	90	Penicillin Tetracycline Oleandomycin/ penicillin combo Placebo	1g, 36months 2g, 36months 2g, 36 months	None available	27% mainly GI upset 27% mainly diarrhoea 20% mainly GI upset 10% mainly diarrhoea	Long term tetracyclines lead to gastrointestinal side effects in 20-30% of cases – predominantly diarrhoea.
Cherniack *et al*, 1959[44]	Randomised	67	Penicillin Tetracycline Oleandomycin/ penicillin combo Placebo	1g, 3-22months 2g, 3-22months 2g, 3-22 months	28% *H. influenzae* 14% *S. pneumoniae* 15% *Staphylococcus spp* 23% yeasts	Exacerbations rates were not significantly affected with penicillin when compared with placebo. Exacerbations rates were lower with tetracyclines - mean exacerbation 1.32 ± 0.3 standard error when compared with placebo 3.36 ± 0.6 P<0.01. Shorter duration of illness with tetracyclines 14.5 ± 2.9 days versus 29.2 ± 5.1 days. There was no significant difference in exacerbation rates with oleandomycin/penicillin when compared with placebo. There was no significant difference in exacerbation rates between tetracycline (1.32 ± 0.3) and the combination oleandomycin/penicillin 1.92 ± 0.3. No significant impact on lung function FEV_1 or FVC, sputum volume or sputum purulence.	This study supports that long term tetracyclines reduce exacerbation frequency.
Dowling *et al*, 1960[45]	Randomised	89	Penicillin Tetracycline	1g, 3-31months 2g, 3-31months	59% *H. influenzae* 5% *S. pneumoniae* 21%	Significant increase in *Pseudomonas spp* with tetracyclines from 19% pre therapy to 43% post therapy. There was a relative reduction in *H. influenzae* and *S. pneumoniae*.	With long term tetracyclines there was a reduction in conventional

			Oleandomycin/ penicillin combination Placebo	2g, 3-31 months	*Staphylococcus spp* 12% *Pseudomonas spp*	Increase in *Klebsiella spp* with penicillin from 25% pre therapy to 54% post therapy but a relative reduction in *S. pneumoniae*. Increase in *Proteus spp* from 14% to 43% with combination Oleandomycin/penicillin therapy but relative reduction in *S. pneumoniae*.	bacteria that would respond to tetracycline e.g. *S.pneumoniae, H.influenzae, S.aureus* but an increase in *P.aeruginosa* that does not respond to tetracyclines.
Currie *et al*, 1990[46]	Randomised	38	Amoxicillin Placebo	3g, b.d, 32 weeks	24% *P. aeruginosa*	The Amoxicillin treated group led to 65% clinical improvement vs. 21% in placebo, p=0.02. Other positive effects in the amoxicillin treated group were: Reduction in 24 hour sputum volume (p<0.05) Less time confined to bed and away from work. Less severe exacerbations but no effect on frequency. Well tolerated apart from two patients intolerant due to drug rash and diarrhoea.	High dose amoxicillin over 8 months led to significant clinical improvement with less exacerbations.
Table 3. Oral antibiotic studies in stable state bronchiectasis							

Author	Study	N	Antibiotic	Dose & Duration	Microbiology	Results	Our Interpretation
Tsang *et al*, 1999[47]	Randomised	21	Erythromycin Versus Placebo	500mg, b.d, 8 weeks	76% *P. aeruginosa* 14% *H. influenzae* 5% *K. Pneumoniae* 5% *E. coli*	Erythromycin group had improved FEV_1, FVC & reduced 24hr sputum vol. No difference in microbial load or pro-inflammatory cytokines	Proof of concept study that macrolide therapy over 8 weeks has a possible anti-inflammatory effect.
Wong *et al*, 2012[48]	Randomised	141	Azithromycin Versus Placebo	500mg, 3x/week, 6 months	13% *P. aeruginosa* 27% *H. influenzae* 3% *S. aureus* 1% *S. pneumoniae* (Only common pathogens reported)	Event-based exacerbations were significantly reduced in the azithromycin group (0.59) compared with the placebo group (1.57), p<0.0001. Median time to first exacerbation in 1 year was longer in the azithromycin group 239 versus 85 days with placebo.	First randomised controlled trial with azithromycin 500mg, three times a week, versus placebo reduced exacerbation in the azithromycin treated group.
Altenburg *et al*, 2013[49]	Randomised	83	Azithromycin Versus Placebo	250mg, o.d, 1 year	11 % *P. aeruginosa* 17 % *H. influenzae* 5 % *M. Catarrhalis* 7.5 % *S. aureus* 4 % *A. fumigatus* 1 % *H. parainfluenzae* 7.5 % *S. pneumoniae* 1 % *X. maltophilia* 1.5 % *S. marcescens* 1 % *E.coli* 1.5 % *E. cloacae*	Median number of exacerbations 0 in azithromycin group compared with 2 in placebo group, p<0.001. %Predicted FEV_1 increased by 1.03 per 3m and %predicted FVC by 1.33 in azithromycin treated patients. GI adverse effects in 40% in the azithromycin group and 5% in the placebo but no discontinuation needed. Macrolide resistance was 88% in the azithromycin group versus 26% in the	Similarly, this study showed azithromycin 250mg, once daily, over a year reduced exacerbations but gastrointestinal side effects are common. Oropharnygeal streptococcus develops significant macrolide resistance and the long term significance of this needs to be explored further.

					2.5 % *A. xylosoxidans*	placebo group.	
Serisier *et al*, 2013[50]	Randomised	117	Erythromycin ethysuccinate Versus Placebo	400mg, b.d, 48 weeks	35% *P. aeruginosa* 20.5% *H. influenzae* 45.35% 'normal flora (no pathogens)'	Erythromycin significantly reduced exacerbations (1.29 vs 1.97/patient/yr for placebo, p-0.003). Subgroup analysis showed a similar trend in patients colonised with *P. aeruginosa*. Sputum volume was reduced in the erythromycin group. % Predicted FEV_1 decline was attenuated with erythromycin. Erythromycin increased the proportion of macrolide resistant streptococci (28% vs. 0.04%, p<0.001).	Erythromycin over 48 weeks at 400mg, twice daily, showed similarly a reduction in exacerbations but an increased the proportion of macrolide resistant streptococci. The long term significance of this needs to be explored further.
Table 4. Long term macrolide therapy studies in stable bronchiectasis							

Author	Study	N	Antibiotic	Dose & Duration	Microbiology	Results	Our Interpretation
Lin *et al*, 1997[52]	Randomised	16	Gentamicin Versus 0.45% saline	40mg, b.d, 3 days	Not recorded	The gentamicin group led to a reduction in microbial load, sputum Myeloperoxidase (MPO) levels and sputum volume. There was improved Borg breathlessness score, peak expiratory flow rates and 6 min walk.	Early proof of concept study showing inhaled antibiotics could reduce microbial burden and improve clinical outcomes.
Barker *et al*, 2000[53]	Randomised	74	Tobramycin Versus Placebo	300mg, b.d, 4 weeks on treatment then 2 weeks off	100% *P. aeruginosa*	The tobramycin group at week 4 led to a significant reduction of sputum bacteria by 4.54 log (10) cfu/g with tobramycin with no change in the placebo group, p<0.01. Tobramycin subjects also showed a 35% eradication rate at week 6, with no eradication in placebo. Tobramycin group had increased cough, wheeze, chest pain and breathlessness.	Inhaled tobramycin over 4 weeks can eradicate *P.aeruginosa* in 35% - an outcome that is very different to the cystic fibrosis population.
Wilson *et al*, 2013[54]	Randomised	124	Ciprofloxacin (DPI) Versus Placebo	32.5mg, b.d, 28 days	54% *P. aeruginosa* 24% *H. influenzae* 20% *S. aureus* 7% *S. pneumoniae* 6% *M. catarrhalis* 4% *K. pneumoniae* 6% *P. mirabilis*	There was a significant reduction in bacterial density for those on ciprofloxacin DPI with total sputum bacterial load at day 28 - end of treatment (EOT) (-3.62 $\log_{10}$ $CFUg^{-1}$ (range -9.78–5.02 $\log_{10}$ $CFUg^{-1}$))	Inhaled ciprofloxacin improved bacterial clearance over 28 days and was well tolerated.

					4% *K. oxytoca*	compared with placebo (-0.27 $\log_{10}$ $CFUg^{-1}$ (range -7.96–5.25 $\log_{10}$ $CFUg^{-1}$)) ($p<0.001$). The counts increased thereafter until they were similar at day 84. 14/40 subjects in the ciprofloxacin DPI group reported pathogen eradication at EOT vs 4/49 in the placebo group ($p=0.001$). 3/60 in ciprofloxacin vs 3/64 in placebo reported bronchospasm, 0/60 vs 5/64 reported cough and 1/60 vs 2/64 reported haemoptysis. No change in FEV_1 or FVC for either group. No significant difference between the groups in sputum volume or colour.	
Drobnic *et al*, 2005[55]	Randomised crossover study	30	Tobramycin Versus Placebo	300mg, b.d, 6 months	100% *P. aeruginosa*	No effect on overall number of exacerbations, pulmonary function or health status. The tobramycin treated group had a reduced number of and length of hospital admissions ($p<0.05$). Significant reduction in *P. aeruginosa* bacterial load ($p<0.05$) Bronchospasm occurred in 10%. No significant increase in bacterial resistance.	Inhaled tobramycin in patients with *P.aeruginosa* over 6 months led to a reduction in severity of admissions and with no impact on bacterial resistance.
Serisier *et al*, 2013[56]	Randomised	42	Ciprofloxacin (DRCFI) Versus Placebo	150mg ciprofloxacin for inhalation + 20mg free ciprofloxacin for inhalation, o.d, 24 weeks (3x 28days on/28days off)	100% *P. aeruginosa*	There was a mean reduction in *P.aeruginosa* bacterial density at day 28 of 4.2 $\log_{10}$ CFU/g with DRCFI compared to a mean reduction of 0.08 with placebo ($p=0.002$). Time to first exacerbation was significantly delayed by DRCFI (134 days) when compared with placebo (58days). DRCFI was well tolerated with fewer respiratory related adverse events than placebo. Main treatment related adverse events included nausea, sinusitis, fatigue, headache and abnormal taste. 3 subjects in each arm	In per protocol analysis inhaled ciprofloxacin led to a delayed first exacerbation in this 6 month study.

						developed an exacerbation – thought not to be related to treatment.	
Orriols *et al*, 1999[57]	Randomised open labelled study	15	Ceftazidime + tobramycin Versus Symptomatic treatment	1g, b.d & 100mg b.d, 1 year	100% *P. aeruginosa*	In the active group the mean number of admissions and length of admission were significantly lower than symptomatic treatment. No change in FEV_1 or FVC for overall exacerbation frequency for either group. No significant increase in bacterial resistance.	This small study supports that long term inhaled antibiotics reduce the severity of exacerbations.
Murray *et al*, 2011[58]	Randomised single blinded study	65	Gentamicin Versus 0.9% Saline	80mg, b.d, 1 year	48.1% *P. aeruginosa* 40.7% *H. influenzae* 7.4% *S. aureus* 3.4% *S. pneumoniae*	The gentamicin group led to 1. Increased ETT 95m 2. Leicester Cough Questionnaire 81% had 1.3U improvement or greater vs. 20% placebo 3. SGRQ 82.5% had 4U improvement or greater vs. 19.2% placebo 4. Increased time to next exacerbation (Gentamicin 120d (87-162) vs. Saline 61.5d (20-7-122.7)) 5. Decreased exacerbations (Gent 0(0-1) vs. Saline 1.5(1-2)) 6. No significant effect on antimicrobial resistance There was however in the gentamicin group, 1. No effect 24hr volume, FEV_1, FVC, FEF25/75 2. 21.9% (7 of 32 patients) reported bronchospasm and received adjunctive nebulised β_2 agonist treatment. Despite this, two patients (6%) required withdrawal from the study (one at month 3 and one at month 6) Treatment needs to be continuous for its on going efficacy.	This 1 year study supports long term inhaled antibiotics reduce total number of exacerbations and time to first exacerbation, Treatment, however, has to be continuous for its ongoing efficacy.
Table 5. Inhaled antibiotic studies in stable bronchiectasis							

DEVELOPING DRUG THERAPIES FOR BRONCHIECTASIS

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Abstract

Introduction: Bronchiectasis is a chronic respiratory condition characterised by cough, sputum production and recurrent chest infections. There are multiple aetiologies, but in up to 50% of patients the aetiology is unknown. The treatment is largely symptomatic with regular chest physiotherapy and antibiotics for infective exacerbations. Research is being directed towards breaking the 'vicious circle' of bronchiectasis with therapies directed at improving mucociliary clearance, treating chronic infection and reducing inflammation in the airways.

Areas covered: This review highlights the current status of bronchiectasis research, summarising reported and ongoing studies of potential therapeutic agents not yet assessed in large trials or licensed for treatment. A literature review was performed on the PubMed database and upcoming trials were sought on the clinical trials.gov website. Only studies from preclinical stages to phase II were included.

Expert opinion: The trials discussed offer insight into potential therapeutic agents for the future and help highlight areas in need of further targeted research. There are promising new anti-infective and anti-inflammatory therapies for more advanced bronchiectasis but phase III studies are needed to investigate these agents further and also to decide at what stage therapy should be implemented.

Article Highlights

- Bronchiectasis has become more commonly recognised with the advent of CT of the chest
- The mainstay of treatment for clinically significant bronchiectasis is chest physiotherapy and antibiotics
- Large randomised controlled trials are lacking in bronchiectasis
- Encouraging data has been published in mucoactive therapies, long term antibiotics and anti-inflammatory therapies
- Larger randomised controlled trials are needed to identify long term effects of such therapies and target groups for long term therapy

1. Introduction

Bronchiectasis is a chronic respiratory condition first described over a century ago, characterised by daily cough and chronic sputum production with a predisposition to recurrent chest infections [I]. The prevalence of bronchiectasis is unknown but it is being recognised and diagnosed more commonly, perhaps because high resolution computer tomography (CT) scanning of the chest, the gold standard for diagnosis, is more frequently used in clinical practice. In the United States, the prevalence ranges from 4/100,000 in young adults to nearly 300/100,000 in those older than 75years [2,3]. Diagnostic CT images show the bronchial diameter to be larger than the adjacent pulmonary artery in affected areas (signet ring sign). The degree of bronchial dilatation increases with severity from cylindrical or tubular bronchiectasis in mild disease to varicose bronchiectasis (focal constrictive areas along the dilated airways) to saccular or cystic bronchiectasis in severe disease [4].

The spectrum of bronchiectasis ranges from mild, moderate to severe. The classification of bronchiectasis is complex and there is a degree of overlap. The severity should be classified based on clinical features and not on radiology. In our clinical experience mild disease is associated with the following clinical features: mucoid sputum production when stable, less than 10mls sputum volume/24hours and limited exacerbations (1 or 2 exacerbations a year) which can usually be treated with oral antibiotics. Moderate disease is usually associated with mucopurulent or purulent sputum when clinically stable but not leading to an excess of exacerbations (2 or less exacerbations a year). Patients with severe disease have frankly purulent

sputum when clinically stable, produce more than 10mls/day and have more frequent exacerbations (≥3/year). These patients can be colonised by more difficult pathogens such as *Pseudomonas aeruginosa*, other enteric Gram negative organisms or MRSA. These patients may require intravenous antibiotics and hospital admission to treat their exacerbations [1,5].

Bronchiectasis has been termed an 'Orphan disease' relating to the relative lack of research in this field when compared to asthma, lung cancer and chronic obstructive pulmonary disease (COPD) [6]. Bronchiectasis is often regarded as a heterogenous disease in view of the multiple aetiologies and associated comorbidities. 53% of cases are idiopathic and the second most common cause is a past respiratory infection (29-42%) [1,7,8].

Cole *et al* coined the phrase 'the vicious circle' when describing the pathogenesis of bronchiectasis [9]. The colonisation of lower respiratory tracts with pathogenic bacteria leads to a predominantly neutrophilic inflammatory response in the airways. Despite this host response there is a failure of bacterial clearance [5,10]. The neutrophil products in particular neutrophil elastase can further damage the mucociliary escalator, predisposing to bacterial colonisation which perpetuates the inflammatory response. Thus the vicious circle is established [9].

Therapies designed to break this circle can be grouped into anti-infective, anti-inflammatory and mucoactive agents. The goals of treatment include reducing cough and sputum volume, reducing sputum purulence, reducing the number of chest infections and improving quality of life (QofL).

This review on developing therapies summarises the trials in preclinical to phase II stages of new drugs being explored for the treatment of idiopathic and post-infection bronchiectasis. It is beyond the scope of this paper to comment on treatment strategies for bronchiectasis due to active allergic bronchopulmonary aspergillosis, active sarcoid, cystic fibrosis and immunoglobulin deficiencies which all have their own disease specific treatments.

A literature search was performed on PubMed with the following keyword searches: 'bronchiectasis' or 'non-cystic fibrosis bronchiectasis' and 'phase I study', 'phase II study', 'antibiotics', 'mucoactive therapy', 'anti-inflammatory therapy'. A keyword search on the clinicaltrials.gov website was performed with 'bronchiectasis.' 127 studies were identified, of which 27 were included.

2. Anti-infective treatment

2.1 Long term antibiotics

There is chronic colonisation of the lower respiratory tract with bacteria in up to 70% of patients with bronchiectasis [5,10]. Up to 26% of bronchiectatic patients can be colonized with *Pseudomonas aeruginosa* [10,11]. This bacterium is associated with more frequent exacerbations, poorer quality of life, deteriorating lung function and increased mortality [12-14]. It has been shown that increasing bacterial load is directly proportional to an increase in the number of outpatient exacerbations and hospital admissions [5]. This in turn reduces quality of life of patients – an important clinical endpoint in bronchiectasis. It is therefore considered to be important to attempt to reduce the bacterial load in the stable state with the aim of reducing exacerbations and improving QofL. One such method is the introduction of long term antibiotics. Current antibiotics in published phase II trials - inhaled ciprofloxacin and tobramycin are displayed in table 1.

Table 1 - Long term antibiotics

Intervention/ Control	Patient information	Inclusion microbiology	Primary endpoint	Outcome CFU/(MIC)	Other outcomes	Study
Ciprofloxacin DPI, 32.5mg, *b.i.d*, days 1-28, n=60 Placebo, *b.i.d*, days 1-28, n=64	• N=124 • Stable for 30days • 1 hospital admission or 2 courses of antibiotics in the last 12 months • Those on long term antibacterial therapy were excluded	Culture positive for pre-defined respiratory pathogens (PA, HI, SA, SP, MC, KP, PM, KO).	Total bacterial density in sputum after 28 days of treatment	Greater mean reduction in CFU/ml during treatment and at end of treatment (EOT) DAY 28 was seen in the ciprofloxacin group p<0.001 Bacterial eradication at EOT in 35% ciprofloxacin group versus 8% placebo group p=0.001. 6 subjects had increased MIC levels >4mg/L at EOT in the ciprofloxacin arm, nil in placebo group	2 treatment emergent serious adverse effects compared to 3 in placebo group	Wilson *et al* [15] Randomised double-blind, 2013
Ciprofloxacin, Dual release for Inhalation (DRCFI), *o.d*, for 3 cycles of 28days on/28days off, n=20 Placebo, *o.d*, for 3 cycles of 28days on/28days off, n=22	• N=42 • 2 or more exacerbations in last year	Ciprofloxacin sensitive *Pseudomonas aeruginosa* at screening in sputum	Change in *P. aeruginosa* bacterial density to end of treatment cycle 1 (day 28)	Significant reduction in bacterial load at day 28 in DRCFI group p<0.001 (modified intention to treat analysis) Failure to culture PA was more frequent in DRCFI group (60% versus 14%) p=0.003 No significant difference in MIC levels	Prolonged time to next exacerbation (134 days versus 58) p=0.046 in a modified intention to treat analysis No difference between the groups at day 28 for FEV_1, QofL or 6minute walk test (6MWT)	Serisier *et al* [16] Randomised double-blind, 2013
Tobramycin (nebulised), 300mg, *b.i.d*, 28 days , n=37 Placebo (1.25mg	• N=74 • No antibiotics within 2 weeks of screening visit	At least 10^4 cfu *Pseudomonas aeruginosa* per gram of sputum	Change in *P. aeruginosa* bacterial density from baseline to	Mean decrease of $4.54\log_{10}$ cfu/g sputum of PA in Tobramycin solution for inhalation(TSI) arm versus a	Reduction in PA was a significant predictor of improved medical condition (subjective	Barker *et al* [17] Randomised Double-blind,

quinine sulphate), b.i.d, 28 days, n=37			week 4	mean increase of $0.02\log_{10}$ cfu/g with placebo ($p<0.01$) Tobramycin resistant strains in 11% in TSI arm versus 3% in placebo arm ($p=0.36$) Eradication of PA in 35% of TSI patients at week 6	analysis: improved or not improved) throughout treatment ($p<0.01$) and at week 6 ($p<0.04$) in TSI arm No difference in FEV_1 in either group but incidence of dyspnoea, wheeze and chest pain significantly greater in TSI arm ($p=0.01$)	2000
Tobramycin (nebulised), 300mg, *b.i.d*, or placebo *b.i.d*, each for 6 months in a crossover fashion with a 1 month washout period in between the cycles	• N=30 • An inpatient 2 week course of intravenous ceftazidime and tobramycin prior to entry	At least 3 sputum cultures in the last 6 months identified *Pseudomonas aeruginosa*	No powering estimation was performed in this study. Efficacy was primarily evaluated by means of number of exacerbations, number and days of hospital admission	Significant decrease in bacterial density with tobramycin ($p=0.038$) No change in bacterial resistance	No difference in the frequency of pulmonary exacerbations Significant reduction in number of hospital admissions ((Mean+/- SD) 0.15+/- 0.37 in tobramycin period versus 0.75+/-1.16 in placebo group) and length of stay in tobramycin period 2.05+/- 5.03 days versus 12.65 +/- 21.8 days ($p<0.047$) No change in FEV_1, FVC or QofL Bronchospasm in 10% tobramycin patients (n=3)	Drobnic *et al* [18] Crossover Double-blind, 2005
Ciprofloxacin, oral,	• N=53	Chronic colonization	Clinical outcome	Significant mean PA density	No difference in clinical	Bilton *et al*

750mg and tobramycin, inhaled, 300mg/5mls, *b.i.d*, 14 days, n=26 Ciprofloxacin, oral, 750mg and placebo, 5mls (1.25mg quinine sulphate), *b.i.d*, 14 days, n=27	• Acute exacerbation	with *Pseudomonas aeruginosa*	assessment at day 21 (test of cure)	reduction in the Cip/Tobra arm (3.67log_{10} versus 1.15log_{10} on day 7 and by 3.25log_{10} versus 0.52log_{10} on day 14 ($p<0.001$)	outcomes between the groups at day 14 or day 21 Significant increase in frequency of wheeze with 50% in cip/tobra versus 14.8% in cip/placebo ($p<0.01$)	[19] Randomised Double-blind, 2006

Pseudomonas aeruginosa (PA), Haemophilus influenzae (HI), Staphylococcus aureus (SA), Streptococcus pneumoniae (SP), Moraxella catarrhalis (MC), Klebsiella pneumoniae (KP), Proteus mirabilis (PM) and Klebsiella oxytoca (KO).

Further phase 3 studies assessing the time to next exacerbation and frequency of exacerbations are underway using inhaled ciprofloxacin. The RESPIRE study (NCT01764841) will randomise patients into one of four groups – 28day on/28 days off ciprofloxacin dry powder for inhalation (DPI), 28day on/28 days off placebo, 14 days on/14days off ciprofloxacin DPI and 14 days on/14days off placebo for 48 weeks. ORBIT-3 (NCT01515007) randomises patients to receiving dual release ciprofloxacin for inhalation (DRCFI) once daily for 28 days on/28 days off therapy for six cycles or to placebo. These studies are currently ongoing.

In addition to the studies shown in table 1 there is an open-label phase II trial recruiting to assess the efficacy of a dry powder for inhalation form of tobramycin (NCT02035488). The main objective of the study is to assess the pharmacokinetics of dry powder tobramycin delivered through the 'Cyclops' inhaler device at different doses and the local tolerability. 8 patients are to be recruited and will be given one dose of tobramycin every week. Doses will increment form 30mg to 60mg then 120mg and finally 240mg. Primary outcome measure includes actual dose delivered.

There is another open label trial ongoing assessing bacterial density as the primary endpoint comparing combined inhaled and systemic tobramycin therapy with systemic therapy alone (NCT01677403). Patients colonised with Pseudomonas aeruginosa will be given either saline or nebulised tobramycin 80mg *b.i.d* for 14 days in addition to systemic therapy (no additional details available). The trial is currently still recruiting and aims to enrol 120 patients.

Tobramycin has also been studied as part of a fosfomycin/tobramycin combination (4:1 w/w) drug (FTI) [20]. Results show this could be a novel combined inhaled antibiotic for patients with bronchiectasis and cystic fibrosis. A phase II study assessed the formulation's activity in *vitro* and in *vivo.* Clinical isolates from cystic fibrosis and bronchiectasis patients were evaluated for MIC, minimum bactericidal content, post antibiotic effect, synergy, spontaneous mutation frequency (SMF), time-kill and in a rat pneumonia model [20]. Results showed the combination drug to have a potent dose dependent bactericidal action against *Pseudomonas aeruginosa* in the rat pneumonia model but tobramycin alone was found to be more active than the combination drug with a lower MIC_{90} (mg/L). For *Pseudomonas aeruginosa* and *Staphylococcus aureus* the SMF was lower for FTI than either of the single drugs alone. FTI had a lower MIC than either tobramycin or fosfomycin against staphylococcus aureus (methicillin resistance detected in 75% of strains) and a comparable MIC_{90} with vancomycin (MIC_{90} of 2 versus MIC_{90} of 1). FTI also demonstrated high activity against *Staphylococcus aureus, Haemophilus influenzae, Klebsiella pneumoniae* and *Escherichia coli* but was poor against *Stenotrophomonas maltophilia* and *Bukholderia cepacia.* Synergy testing did not show any antagonism between the two drugs. Dual activity against both Gram positive and Gram negative bacteria make it an attractive choice for patients colonised with multiple pathogens and may be useful in attenuating the development of drug resistant organisms.

Another antimicrobial currently being investigated for patients with bronchiectasis is inhaled amikacin via nebuliser. A multi-national phase II trial assessing the safety and tolerability of Arikace-a liposomal form of amikacin has been completed (NCT00775138). Patients chronically colonised with *Pseudomonas aeruginosa* (n=64) were recruited. The double blind trial randomised patients to nebulised once

daily dosing of either Arikace 280mg, Arikace 560mg or placebo for 28 days. Co-primary endpoints assessed treatment emergent adverse events and pulmonary function abnormalities. Secondary endpoints included time to next exacerbation, quality of life and bacterial density. An interim analysis reported no concerns regarding safety and tolerability but full results have not been published yet [21].

2.2 Anti-viral therapy for viral exacerbations

The role of viruses in bronchiectasis exacerbations is poorly understood. The British national quality standards for bronchiectasis advise physicians to provide patients with a self-management plan to enable them to take ownership and responsibility for their condition [22]. They also advise an annual influenza vaccine and to avoid contact if possible with people known to have a viral infection to prevent viral induced exacerbations [22]. This is based on expert consensus from clinicians managing bronchiectasis as there are no completed studies to date investigating the role of viruses and anti-viral treatment in bronchiectasis exacerbations. Studies are needed to assess the clinical efficacy of antiviral therapy in bronchiectasis exacerbations to help guide management. A phase 1 study (NCT01113034) assessing the safety of antiviral agent DAS181 in patients with bronchiectasis was underway but further information regarding its current status is not available. DAS181 is a fusion protein of the sialidase catalytic domain of *Actinomyces viscosus* and the anchoring domain of the human amphiregulin. It's mechanism of action consists of cleaving the sialic acid receptor on the cells surface of host cells which influenza virus requires to infect cells [23,24]. This novel action could make it a potent inhibitor of seasonal and emerging influenza strains [25].

2.3 Preventative therapy for exacerbations

In addition to the above studies there has been interest in immunostimulating agents. These compounds consist of antigens from several bacterial strains and are designed to stimulate an immune response. OM-85 (also known as bronchoVaxom) consists of extracts of eight different bacterial species (*Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae and Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans* and *Neisseria catarrhalis*). It is thought to directly activate lung macrophages, enhance antigen presentation, and encourage differentiation of CD4 T lymphocytes and B lymphocytes [26]. The agent has been studied in COPD and was found to significantly reduce exacerbations [27-29]. A randomised double blind multicentre placebo-controlled trial is planned looking at the effect on exacerbations in bronchiectasis but recruitment has not yet commenced (NCT01968421).

3.0 Mucoactive agents

Hyperosmolar agents, sometimes referred to as 'mucoactive' therapies are designed to enhance mucus clearance by altering its physical structure, making it less viscous and more mobile. Therapies designed to enable easier expectoration of sputum aim to break the vicious circle by preventing the infection of stagnant mucus in the lower airways.

N-acetylcysteine (NAC) has been investigated as a mucolytic therapy in COPD but to date no evidence exist in the field of bronchiectasis. NAC is thought to disrupt the

mucus gel structure by altering the degree of crosslinking or interactions between molecules in the sputum [30]. Zheng *et al* conducted a randomised trial and propose a 600mg twice daily regime can reduce the rate of exacerbations in moderate and severe COPD patients [31]. Whilst further work is needed before it is incorporated into clinical practice [32], the potential for NAC to be used in other respiratory conditions like bronchiectasis should be considered. A randomised control trial is currently ongoing where the researchers aim to recruit 120 bronchiectasis patients to either NAC 600mg twice daily for 12 months or placebo (NCT02088216). The primary endpoint examines exacerbations of bronchiectasis.

4.0 Anti-inflammatory agents

The inflammation caused by persistent bacterial infection and colonisation further propagates the vicious circle. This part of the circle naturally lends itself to being targeted by anti-inflammatory therapies.

Statins have been used in cardiovascular medicine to lower cholesterol and have also been associated with reduced mortality rates in patients with influenza virus [33]. Their pleotropic effects, in particular, their anti-inflammatory properties have made them a potential therapeutic agent in bronchiectasis. Of note there is reduced neutrophil inflammation in healthy volunteer studies exposed to lipopolysaccharide [34]. Mandal *et al* recently published a randomised controlled trial of high dose atorvastatin (80mg) versus placebo in bronchiectasis (see table 2) [35]. The same group have gone on to assess the use of atorvastatin in patients with *Pseudomonas aeruginosa* in a crossover trial assessing cough as the primary endpoint (NCT01299194). Patients will receive 80mg atorvastatin or placebo for 3 months followed by a 6 week wash out period. They will then crossover to the other arm for a further 3 months. The study will also assess the effect on cough. This trial has completed recruitment but results have not yet been published.

Theophylline forms part of the stepwise treatment for asthma as outlined in the NICE guidelines [36,37]. The mechanism of action of theophylline is not fully understood but it thought to have some anti-inflammatory effects by inhibiting phosphodiesterase (inhibits TNF✔, inhibits leukotriene synthesis, reduces inflammation and innate immunity) in addition to antagonising adenosine receptors and inhibiting TGF-beta mediated conversion of pulmonary fibroblasts. Theophylline may be useful in patients with steroid resistance as it is able to restore histone deacetylase levels and enhance the anti-inflammatory effects of steroids [38]. There have not yet been any large trials in bronchiectasis but there are two small randomised parallel studies currently recruiting patients in bronchiectasis. The first study assesses the safety and efficacy of 24 weeks of theophylline 100mg, twice daily against placebo (NCT01684683). A further randomised study investigating the role of theophylline with low dose formoterol-budesonide over a 24 weeks period is also recruiting for patients with bronchiectasis (NCT01769898). The primary outcome for both studies assesses quality of life as measured by the SGRQ. Similarly, Roflumilast - a phosphodiesterase type 4 inhibitor has shown some efficacy in reducing neutrophilic inflammation in COPD patients but has not yet been studied in bronchiectasis [39]. Roflumilast has been licensed in COPD and could be a potential anti-inflammatory agent in bronchiectasis but randomised controlled trials are required first. A phase II study is planned in symptomatic

bronchiectasis patients but status information regarding this trial is not available (NCT01580748).

Non-steroidal anti-inflammatory drugs (NSAIDS) have been investigated for their anti-inflammatory effects in bronchiectasis (see table 2). The mechanism of action in the study by Llewellyn-jones showed reduced resident neutrophil chemotaxis and therefore this agent may be useful in bronchiectasis by reducing airway neutrophil inflammation[40].

Table 2 - Anti-inflammatory therapies

Intervention/ Control	Patient information	Inclusion microbiology	Primary outcome	Outcome CFU/(MIC)	Other outcomes	Study
Atorvastatin, 80mg, *o.d*. for 6 months, n=30 Placebo, *o.d.* for 6 months, n=30	• Clinically significant bronchiectasis • 2 or more chest infections in preceding year • Smokers, >15 year pack history, ex-smokers of <1 year were all excluded	Patients with chronic colonisation with *Pseudomonas aeruginosa* were excluded	Reduction in cough from baseline to 6 months, as measured by the LCQ	Chronic colonisation increase from 57% to 63% with atorvastatin Chronic colonisation remained at 40% in placebo group No significant difference in bacterial load at 6 months in either group	Significant improvement in cough (p=0.01) in the atorvastatin group. 33% in atorvastatin group had an adverse event versus 10% in placebo group (p=0.02) FEV_1 and FVC unchanged Exercise capacity improved by 35m in the atorvastatin group Serum interleukin 8 and CRP fell from baseline levels with atorvastatin 8/24 had ≥2 exacerbations and 5/24 had ≥3 exacerbations with atorvastatin versus 16/29 and 10/29 in placebo group respectively	Mandal *et al,* [35] Randomised control trial, 2014
Indomethacin, 2mls of aerosolized preparation containing 1.2 g/ml, *t.i.d*, 14 days, n=13 Placebo, *t.i.d,* 14	• N=25 • Bronchorrhoea for 4 weeks prior to study Chronic bronchitis, (12) diffuse panbronchiolitis (5),	(17) PA, (3) HI, (1) SA	Primary endpoint not specified in paper	• No change in total bacterial CFU/g or in bacterial flora in either group	No significant difference in FEV_1 or FVC in either group Breathlessness assessed by Borg's score improved with indomethacin only	Tamaoki *et al,* [41] Randomised controlled trial, 1992

days, n=12	bronchiectasis (8)				(from 7.1 ±0.5 to 4.5±0.4, p<0.01) 2 patients in the indomethacin group developed dry mouth but hypotension or bronchoconstriction was not observed Sputum weight significantly reduced from 189±19g to 95±21g/day at day 14 in the indomethacin group (p<0.001)	
Indomethacin, 25mg, *t.i.d*, 28 days, n=9 Indomethacin, 25mg, *t.i.d*, 14 days, n=8	• N=9 clinically stable bronchiectasis • No inhaled or oral steroids in last 3 months • N=8 healthy volunteers	(7) HI, (5) BC, (3) SP, (1) PA, (1) PM from the 9 bronchiectasis patients (patients grew more than 1 organism)	Primary endpoint not specified in paper (lab based study)	• No significant difference in total sputum bacterial load, no change in 12hour sputum volume in sputum	No adverse effects recorded, no change in sputum characteristics or peak flow recorded and no exacerbations during treatment Significant reduction in peripheral neutrophil chemotaxis to 10nmol/L FMLP (p<0.0001) at day 28, returning to baseline at day 63 Significant reduction in fibronectin degradation by resting and stimulated neutrophils in bronchiectasis patients at day 14 and day 28,	Llewellyn-jones *et al,* [40] Open labelled, 1995

					returning back to baseline at day 63 ($p<0.001$) Similar neutrophil results in healthy controls after 14day treatment No change in superoxide generation, intracellular elastase or myeloperoxidase in bronchiectasis samples	
AZD9668, 60mg, *b.i.d*, 4 weeks, n=22 Placebo, *b.i.d,* n=16	• Idiopathic or post-infective bronchiectasis • Clinically stable for 6 weeks prior to entry into study	9 with non-*Pseudomonas* organisms, 9 with *Pseudomonas* in AZD9668 group	No powering estimation was performed for this study	• No information supplied	Significant changes defined a *priori* as $P<0.1$ No change in sputum neutrophils with AZD9668 FEV_1 improved by 100mls (P=0.006) and slow vital capacity improved by 130mls (P=0.079)with AZD9668 Plasma IL-8 reduced with AZD9668 (P=0.085) Post waking sputum IL-6 reduced with AZD9668 (P=0.098) No significant difference in QofL, sputum weight or other lung function parameters	Stockley *et al*, [42] Randomised control trial, 2013

					AZD9668 was well tolerated with commonest side effect of headache (7/22 in AZD9668 versus 2/16 in placebo group)	
Beta carotene, oral, 0.69 ± 0.19 mg kg^{-1} *t.i.d* with meals for cystic fibrosis and bronchiectasis patients only, 6 months	• Cystic fibrosis (CF) n=18, bronchiectasis (BE) n=15, healthy children n=15 • Conditions where reactive oxygen species have been implicated were excluded (cirrhosis, hepatitis, diabetes mellitus, corticosteroid therapy or insulin therapy	Not specified	No primary endpoint specified, explorative study	• Not specified	Vitamin E (VE) levels were significantly lower than healthy volunteers at baseline with CF (p=0.001) and BE (p=0.0001) Beta carotene (BC) levels were significantly lower in CF group than healthy volunteers at baseline (p=0.008) CF and BE has significantly higher malondialdehyde (MDA, biomarker of lipid peroxidation due to free radical oxidation) than healthy controls at baseline (p=0.0001) Post treatment VE and BC increased in both CF (p=0.007, p=0.001) and BE (p=0.008, p=0.001) Post treatment reduction in TNF ✔ and MDA in both	Cobanoglu *et al*, [43] Open labelled, 2002

					CF (p=0.022, p=0.001) and BE (p=0.035, p=0.015) Post treatment vital capacity improved in BE (P=0.02), FEV_1 and FEV_{25-75} improved in CF (P=0.016, P=0.017 respectively)	

Haemophilus influenzae (HI), Pseudomonas aeruginosa (PA), Streptococcus pneumoniae (SP), Haemophilus parainfluenzae (PI), Moraxella catarrhalis (MC), Stenotrophomonas maltophilia (SM), Klebsiella pneumoniae (KP) and Candida albicans (CA), Staphylococcus aureus (S)A, Branhamella catarrhalis (BC*,) Proteus mirabilis (PM).* N-formyl-methionyl-leucyl-phenylalanine (FMLP)

Neutrophil elastase inhibitors are thought to have potential in reducing the inflammatory response seen in the airways of patients with bronchiectasis. In bronchiectasis there is excess neutrophilic airways inflammation and free elastase is found in patients with more advanced bronchiectasis. Neutrophil elastase inhibition offers a potential therapeutic intervention. To date, there has been one phase II trial investigating the compound AZD9668 – an oral neutrophil elastase inhibitor (see table 2) [42].

The generation of oxygen free radicals is a part of the neutrophilic inflammatory response and is known to damage airways. It has therefore been postulated that the imbalance between oxidants and antioxidants may play a role in bronchiectasis. To investigate this further Cobanglu and colleagues explored the effect of beta carotene in children with cystic fibrosis (CF), bronchiectasis and healthy controls [43].

Novel treatments are being sought to break the 'vicious circle' of bronchiectasis and some of the newer agents include AZD5069 (CXC chemokine receptor 2 antagonist CXCR2). Its mechanism of action includes reducing calcium flux and reducing chemotactic response to known chemoattractants. AZD5069 had undergone phase I studies to understand its safety and efficacy (NCT00953888) and the change in pharmacokinetic profile when co-administered to healthy volunteers with ketoconazole (NCT01735240). A subsequent phase II study (NCT01255592) enrolled 83 bronchiectasis patients in a randomised placebo controlled trial to receive either AZD5069 twice daily for 28 days or placebo. The trial has concluded but results have not yet been published.

5.0 Conclusion

The vicious circle of bronchiectasis needs to be broken in order to improve symptoms, prevent recurrent infections and halt any potential progression of the disease. A number of new therapies are in the pipeline targeting not only the bacterial burden in the lower airways but also the excessive neutrophilic inflammatory response seen in patients with bronchiectasis. Ultimately phase III trials are needed before these agents can become licensed for routine use in bronchiectasis.

6.0 Expert commentary

Bronchiectasis was first described over a century ago and despite this there is a lack of large randomised controlled trials compared to other chronic respiratory conditions. The heterogeneity of this condition and associated co-morbidities makes managing this condition challenging. We have focused the review on idiopathic and post-infective bronchiectasis, the commonest aetiologies found in bronchiectasis, which in our opinion is managed similarly. The aims of treatment for patients with clinically significant bronchiectasis are to reduce cough, sputum volume, sputum purulence, number of exacerbations and to improve quality of life.

The management of bronchiectasis should be based on the clinical impact for each individual patient. For patients with mild disease treatment should be focused on prevention of exacerbations. This includes daily chest clearance using the active cycle breathing technique or alternatives recommended by the respiratory physiotherapist, if unable to carry out the active cycle breathing technique. For younger patients we would recommend positive expiratory pressure devices such as the Acapella®. We would recommend an annual influenza vaccination and the

pneumococcal vaccination every 5 years. The conjugate pneumococcal vaccine in our opinion offers potential immunological therapeutic advantages over the traditional polysaccharide vaccine but requires further study. Immunostimulating agents are an exciting future therapeutic strategy but further studies are needed before this can be implemented into clinical practice. This group of patients have limited exacerbations and exacerbations should be treated promptly with antibiotics as per sputum culture and sensitivities. In our opinion there is no need in this group to receive mucoactive therapies or long term anti-inflammatory or anti-infective therapies.

In our view therapy for moderate clinical disease therapy should be targeted at improving symptoms and preventing disease progression. In our opinion these patients may benefit from muco-active therapies. The strongest evidence exists for hypertonic saline [44-46] and mannitol [47-52] which increase sputum expectoration and promote airway clearance. We would recommend using hypertonic saline (7%) in patients that have difficulty in achieving airways clearance with active cycle breathing technique alone. We would recommend reserving the use of inhaled mannitol until the results are available from the ongoing phase III studies. Anti-inflammatory agents are an exciting therapeutic option which may improve symptoms and prevent disease progression. Recently, we have seen the publication of large trials assessing the use of macrolides as a potential anti-inflammatory therapy to reduce exacerbation frequency [53-55]. We believe that the side effect profile of macrolides in this group of patients mitigates the potential therapeutic advantages of reducing exacerbations. The side effect profile we are concerned with are the increased pneumococcal resistance to macrolides, the adverse effects on hearing and balance, the gastro-intestinal side effects and the potential risk of macrolide resistant non-tuberculous mycobacteria. In patients with bronchiectasis there is excess neutrophil airways inflammation with free elastase activity and high levels of chemo-attractants such as interleukin 8, leukotriene B4 and Complement 5A in the airways. Our view is that the neutrophil elastase inhibitors, CXCR2 antagonists and statins are the most promising therapeutic targets as they target the neutrophil airways inflammation. The best evidence exists for statins as they have been shown in a preliminary study to improve neutrophil apoptosis and reduce cough but is not sufficient currently to put into routine clinical practice [35]. The benefit of statins over macrolides is it not being an antibiotic and therefore if it can be tolerated it is a safer long term strategy. Ultimately larger randomised controlled trials are needed. In our practice anti-inflammatory therapies are not currently used for this group but probably should be in the future when more evidence exists.

For severe bronchiectasis we would recommend long term antibiotics. The rationale for long term antibiotic treatment is to reduce bacterial burden in the airways. Consequently this would reduce the number of exacerbations and improve quality of life. This was shown in the 12 month phase III trial of nebulised gentamicin [56]. The study showed gentamicin was efficacious in reducing the number of exacerbations, improving quality of life, increasing time to next exacerbation, reducing sputum volume, reducing sputum purulence and reducing bacterial load but reported all markers returned to baseline after 3 months off treatment [56]. The study by Haworth *et al* [57] showed that nebulised colomycin continuously for 6 months reduced time to next exacerbation only if patients complied with therapy. Studies to date using cyclical therapy (using inhaled aztreonam) on and off have

failed to show clinical benefit [58]. In our opinion inhaled antibiotics should be used in patients chronically infected with *Pseudomonas aeruginosa* with 3 or more exacerbations per year. In fully sensitive *Pseudomonas aeruginosa* we would use nebulised gentamicin as first line in view of its proven efficacy and low cost. As second line we would use colomycin and third line tobramycin. We use these agents continuously for life but monitor the gentamicin levels in serum, hearing and renal function and sputum sensitivities.

We use long term oral therapy for patients with severe bronchiectasis (three or more exacerbations per year) and chronic infection with other potential pathogenic organisms except *Pseudomonas aeruginosa*. The commonest pathogen is *Haemophilus influenzae*, and we treat with long term amoxicllin if it is a beta lactamase negative producing organism. We treat these patients long term but monitor side effects, sputum culture and sensitivities and adjust long term treatments as required.

If there is failure despite this treatment we would consider long term anti-inflammatory treatment in addition. We would use long term azithromycin in view that the long term benefits outweigh the disadvantages in this group of patients. We, however, send three sputum samples for mycobacterial culture first and only use in patients with no evidence of non-tuberculous mycobacteria. This treatment would be continued long term as long as there were no side effects that require treatment cessation. We would use macrolides before other potential anti-inflammatory therapies in view that these are the only treatments that have been shown to be of benefit in international trials.

We reserve regular intravenous antibiotics, administered in 8 weekly cycles, for those patients unresponsive or intolerant to the above therapies and have 5 or more exacerbations per year. The only study to date on regular intravenous therapy reported patients feel better and require less antibiotic therapy overall [59].

Originally a lot of therapies were based on those used for cystic fibrosis but we have learnt a lot from the inhaled DNase study [60] that treatments that work in cystic fibrosis can be harmful in non-cystic fibrosis bronchiectasis. In the next five years we hope to see multiple phase III clinical trials using different inhaled antibiotic therapies and multiple phase II trials assessing anti-inflammatory therapies so these treatments can become licensed for patients with bronchiectasis. The ultimate challenge will be to decide when we should institute both anti-inflammatory and anti-infective therapies and in which patients. In our opinion if we target people earlier with moderate disease we will hopefully improve symptoms and halt disease progression. This review has focussed on chronic management but another challenge is defining exacerbations and investigating how they should be treated. Currently we treat with oral antibiotics as first line and if this fails proceed to intravenous antibiotics using therapies based on sputum cultures and sensitivity patterns for 14 days. There are ongoing studies addressing optimal length of therapy (NCT02047773) which is another fertile area for future research.

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Expert Opinion on Investigational Drugs

Inhaled or nebulised Ciprofloxacin for the maintenance treatment of bronchiectasis

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ABSTRACT
Introduction: Inhaled or nebulised antibiotics are a major topic of ongoing research interest in reducing exacerbations in bronchiectasis. There are no licenced inhaled or nebulised antibiotics currently in bronchiectasis.
Areas covered: Inhaled or nebulised ciprofloxacin as a long-term treatment in bronchiectasis.
Expert opinion: Results from the Phase III ongoing trials on inhaled or nebulised ciprofloxacin will be key for the outcome of the drugs but additionally, cost-effectiveness and longer-term studies will be necessary to determine the viability of the drug. Head to head studies are needed to decide on the optimum inhaled or nebulised antibiotic and their place with or without long term macrolide therapy. It is also important to determine what treatment is viable for acute exacerbations due to *P. aeruginosa*. Ciprofloxacin is the only currently available oral agent for exacerbations due to *P. aeruginosa*. The concern is that using inhaled or nebulised ciprofloxacin will prevent the use and efficacy of its oral equivalent, by developing resistance.

Drug summary

Drug name: Ciprofloxacin
Phase: Phase III trials results awaited.
Indication : For patient with bronchiectasis that suffer multiple exacerbations (3 or more a year) chronically infected with *Pseudomonas aeruginosa*
Pharmacology: Fluoroquinolone, strongly active against anaerobic, gram-negative bacilli e.g. *Pseudomonas aeruginosa*, some gram-positive action.
Method of action: inhibits DNA gyrase and topoisomerase IV inhibiting cell division.
Route of administration: Inhaled and nebulised formulations discussed.
Ciprofloxacin has equal bioavailability orally and intravenously.
Chemical formula: $C_{17}H_{18}FN_3O_3 \bullet HCl \bullet H_2O$
Pivotal trials: Wilson et al, Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis: A phase II randomised study. Eur. Respir. J. 2013;41:1107-1115.
Serisier et al, Inhaled, dual release liposomal ciprofloxacin in non-

cystic fibrosis bronchiectasis (ORBIT-2): a randomised, double-blind, placebo-controlled trial. Thorax. 2013;68:812-817.
Phase III trials results awaited.

1.0 Introduction

1.1 Bronchiectasis

Bronchiectasis is an acquired irreversible bronchopulmonary disease where bronchial walls in the lung are permanently inflamed and dilated [1]. This leads to what is known as a 'vicious cycle' [2] between chronic bacterial infection and immune dysregulation. The damaged bronchi lead to debilitation of the mucociliary apparatus and therefore an accumulation of mucus, a favourable environment for bacteria which, in turn, results in increased bacterial infections. This further damages the bronchi by eliciting an inflammatory response and the cycle is perpetuated [3]. *Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Moraxella catarrhalis* and *Streptococcus pneumoniae* are the most commonly cultured pathogens in the sputum of patients with bronchiectasis. *P. aeruginosa* is associated with more severe disease, more rapid decline in forced expired volume in 1 second, increased hospitalisation and worse quality of life. It has also been associated with reduced survival.

Patients have recurrent cough, sputum production and recurrent respiratory tract infections. The mainstream of treatment is chest physiotherapy, annual influenza vaccination and prompt treatment of infective exacerbations. Short term antibiotics for exacerbations are part of routine practice and prescribed based on recent microbiology. Ciprofloxacin is currently the only oral agent active against *P. aeruginosa*. Long-term antibiotics are considered if having three or more exacerbations per year but there are no licensed long-term antibiotics to date for use in bronchiectasis [4]. Inhaled or nebulised antibiotics are an attractive option as it targets high concentration treatment to the airways with the long term aim of chronic bacterial suppression and limits systemic toxicity. Phase 3 trials have been completed for inhaled or nebulised ciprofloxacin so this review will therefore focus on studies to date on inhaled or nebulised ciprofloxacin.

1.2 Overview of the market

Currently there are no licensed long term inhaled or nebulised antibiotics for use in bronchiectasis due to a lack of long-term randomised double blind control trial data. Commonly used unlicensed agents include nebulised gentamicin, tobramycin and colomycin [5,6]. There are increasing interest in other inhaled/nebulised antibiotics such as aztreonam, amikacin and ciprofloxacin [6]. Competitive agents are the long term oral macrolides (mainly azithromycin and erythromycin) showing efficacy in reducing exacerbations [7-10].

2.0 Introduction of the compound

Ciprofloxacin is a broad-spectrum antibiotic compound from the fluoroquinolone class. It is mainly active against anaerobic Gram-negative bacilli, in particular *Pseudomonas aeruginosa* but weakly active against Gram-positive bacteria [11].

Ciprofloxacin is currently available routinely as an oral or intravenous (IV) antibiotic as it has equal bioavailability. However, these routes of administration are

not as efficient in achieving the desired pulmonary concentration at the site of infection as are inhaled or nebulised formulations. Inhaled or nebulised ciprofloxacin has been developed and tested to improve intrapulmonary drug delivery for long term treatment. This should also minimize systemic exposure to the antibiotic, which would subsequently diminish any risk of systemic toxicity [12].

2.1 Pharmacodynamics

Ciprofloxacin's mechanism of action is focussed on the inhibition of DNA gyrase and topoisomerase IV, key components involved in the relaxation of positively supercoiled DNA [13] and its separation respectively, thereby inhibiting cell division [11].

2.2 Pharmacokinetics and metabolism

Orally administered ciprofloxacin is well-absorbed and distributed through the body fluids and tissues with a bioavailability of 70%. Its half-live is relatively short among fluoroquinolones and range between 3 and 5 hours, requiring a dose every 12h [11]. On the other hand, inhaled formulations using liposomal release provide a way of extending the half-life of the compound to 10.5h and thereby supporting single dose per day regime [14]. Ciprofloxacin is eliminated by renal excretion and therefore dosage adjustment is necessary for patients with creatinine clearances lower than 50 ml/min [11].

3.0 Clinical efficacy

There are two main forms of inhaled or nebulised ciprofloxacin undergoing phase III trials for treatment of bronchiectasis patients: dry powder for inhalation (DPI) and as dual-release ciprofloxacin for inhalation (DRCFI), respectively [12].

3.1 Dry Powder for Inhalation Ciprofloxacin (DPI)

Ciprofloxacin DPI refers to aerosolized ciprofloxacin spray dried through the PulmoSphere™ drying process [15]. This method coats the ciprofloxacin core with a dry phospholipid "shell" that quickly disperses upon entering the lung, exposing thereby the ciprofloxacin crystals. Phase I studies of this form of ciprofloxacin assessing its pharmacodynamics and pharmacokinetics revealed achievement of high pulmonary concentrations along with low systemic exposure [16].

A phase II randomized, placebo-controlled, double-blind study was conducted. It included bronchiectasis patients that were either idiopathic or had post infectious bronchiectasis with positive cultures of at least one of the following pathogens: *P. aeruginosa, H. influenzae, M. catarrhalis. S. aureus, S. pneumoniae, S. maltophilia,* Enterobacteriaceae or *Achromobacter xylosoxidans*. They were divided into two arms, one received ciprofloxacin DPI 32.5mg (n=60) and the other one, the corresponding placebo (n=64) dispensed using a T-326 inhaler twice daily for 28 days and followed up for another 56 days after treatment [17]. The study met its primary outcome showing a statistically significant reduction in bacterial load in the ciprofloxacin DPI cohort compared with the placebo one with a reduction equal to 3.62 log colony forming units/ml at 28 days. There were trends to an improvement in quality of life with ciprofloxacin but this improvement did zznot reach statistical or clinical significance. The study was too short to show an impact on exacerbations. Following the promising outcomes of phase I and II of ciprofloxacin DPI, phase III

clinical trials (RESPIRE-1 and RESPIRE-2) have been designed to assess time to first exacerbation and mean exacerbations as the primary outcome and results are awaited. Both studies are international prospective, parallel-group, randomized, double-blind, multicentre, placebo-controlled trials with a timeframe of 48weeks and a 8-week follow-up period after end of treatment [18]. These will consist of two different cycle patterns of 28 days on/off drug for 6 cycles or 14 days on/off for 14 cycles with an inhaled twice daily regimen of 32.5mg regimen. The phase II trial showed increased levels of resistance towards ciprofloxacin at the end of treatment but an improvement in susceptibility after a period off treatment. The on/off design of the study is used to reduce the probability of developing resistance with long term antibiotics.

3.2 Dual Release Ciprofloxacin for Inhalation (DRCFI)

While inhaled formulations of ciprofloxacin proved to be efficient to ensure localised delivery of the antibiotic, one pitfall is that the residence time is relatively low as the compound gets rapidly absorbed in the system [19]. This rationale led to the development of a liposomal formulation that allows a reduced frequency of dosing. This may increase the likelihood if patient compliance [19].

Preliminary studies of liposomal ciprofloxacin (Lipoquin) administered to rabbits as aerosols showed a half-life of the compound ranging from 8.3 to 10.2h in the lung, consistent with results in mice and significantly higher than values obtained through intravenous delivery [20]. Based on these results, Lipoquin®, a liposomal ciprofloxacin compound developed by Aradigm Corp made of a bilayer composition combining HSPC and cholesterol was subjected to clinical trials [21]. Furthermore, another compound made up of a 1:1 mixture of Lipoquin (50mg/mL) and free ciprofloxacin (FCI, 20mg/mL) called Pulmaquin® also developed by Aradigm Corp. was also subjected to clinical trials [21].

A first phase I trial involving 20 health volunteer was set up to evaluate the safety, tolerability and pharmacokinetics of Lipoquin looking at a daily single dose given to the cohort for one week. The compound was generally well tolerated with no serious adverse events recorded and showed pharmacokinetics results consistent with the expected values. This study set the bases for the use of a once-daily inhaled dose of ciprofloxacin in subsequent trials.

An initial open-label trial involving 36 patients from multiple centres and carried over a 4-week period compared the administration of two different doses of inhaled Lipoquin to bronchiectasis patients chronically infected with *P. aeruginosa*. Results demonstrated a significant mean decrease of *P. aeruginosa* colony forming units/g compared to baseline during and one week after the 28-days treatment period. However, there was no statistically significant difference between the two groups (3mL and 6mL) but the 3mL group did not report any serious drug-related adverse reactions whereas one possible drug-related adverse reaction was reported for the 6mL dose group. There was no difference in the number of respiratory treatment emergent adverse events between the active and placebo groups.

Following the encouraging results, the combinatory formulation of liposomal ciprofloxacin with free un-encapsulated ciprofloxacin was also evaluated to establish whether any further clinical benefits could be added from a more rapid peak of ciprofloxacin concentration. The mixture composed of 3mL of Lipoquin and 3mL of free ciprofloxacin proved to be safe and well tolerated and was later named Pulmaquin.

Subsequent series of trials were named ORBIT: Once daily Respiratory Bronchiectasis Inhalation Treatment. The first one, ORBIT-1 consisted of a Phase 2b international, double-blind, placebo-controlled trial of Lipoquin that involved a total of 95 adult bronchiectasis patients with chronic infection with *P. aeruginosa*. Two doses were included in the study 100mg (2mL) and 150mg (3mL) ciprofloxacin as well as their corresponding placebos, respectively. The primary end-point of mean change of *P. aeruginosa* colony forming units/g from baseline to day 28 was achieved. Both group showed statistically significant mean reduction although there was no significant difference between the 2mL and 3mL Lipoquin doses. Therefore, the 3mL dose of Lipoquin was confirmed as the optimal liposomal dose [22].

The ORBIT-2 clinical trial was a Phase 2b international, double-blind, placebo-controlled, multi-centre study expanding over 168 days looking at the 6mL (210mg) Pulmaquin formulation and involving a total of 42 adult bronchiectasis patients with chronic infection with *P. aeruginosa*. The primary end-point of statistically significant mean reduction of colony forming units/g (28 days) was achieved and Pulmaquin was found to be well-tolerated by the cohort [22]. The Pulmaquin treated group resulted in a mean (SD) 4.2 (3.7) log10 CFU/g reduction in P aeruginosa bacterial density at day 28 (vs -0.08 (3.8) with placebo, p=0.002). There were fewer exacerbations requiring antibiotic treatment (40% versus 77%, p=0.027) and longer time to first exacerbation (134days versus 58days p=0.046 per protocol or p=0.057 modified intention to treat analysis) in the Pulmaquin treated group. There was no difference in FEV_1, SGRQ score or 6minute walk test between the Pulmaquin treated and placebo groups.

Based on the evidence, Phase 3 ORBIT-3 and ORBIT-4 trials have been designed and are currently ongoing involving approximately 255 patients each and both are randomized, placebo-controlled, international, multi-centre studies. They are organised as 6 cycles of 28days on/28days off treatment followed by a final 28 day open-label extension with all patients receiving Pulmaquin. The primary end-point was determined as the time to first exacerbation with secondary end-points looking at further parameters such as number of pulmonary exacerbations, number of severe pulmonary exacerbations and quality of life [22,23].

Table 1: Summary of completed and ongoing trials of ciprofloxacin in bronchiectasis based on the data available on https://clinicaltrials.gov/

Intervention [Study]	Phase	Primary Outcome Measures	Secondary Outcome Measures	Arms and assigned interventions	Outcome
Radiolabelled Ciprofloxacin Dry Powder for inhalation (DPI) (Cipro Inhale, BAYQ3939)	I	Ciprofloxacin pharmacokinetics, including lung deposition [timeframe: within 24h of treatment]	Adverse events collection [timeframe: follow-up 2 weeks]	• Ciprofloxacin 32.5mg single powder dose of 99m Tc labelled ciprofloxacin PulmoSphere inhalation powder inhaled by healthy subjects • Ciprofloxacin 32.5mg single powder dose of 99m Tc labelled ciprofloxacin PulmoSphere inhalation powder inhaled by healthy subjects under charcoal block • Ciprofloxacin 32.5mg single powder dose of 99mTc labelled ciprofloxacin PulmoSphere inhalation powder inhaled by COPD patients • Ciprofloxacin 32.5mg single powder dose of 99mTc labelled ciprofloxacin PulmoSphere inhalation powder inhaled by bronchiectasis patients	Single dose 32.5mg of ciprofloxacin DPI safe and well tolerated; systemic exposure considerably smaller than parenteral treatment, $t_{1/2}$ clearance and volume of distribution greater than values reported for oral and IV ciprofloxacin.
Ciprofloxacin DPI (Cipro, BAYQ3939)[13]	II	Change from baseline in total bacteria load in the sputum at the end of treatment [day 29]	Change in FEV_1 and FVC from baseline; time to exacerbation with antibiotic intervention; effect on HRQoL (using both the SGRQ and CRQ-SAS); change in C-reactive protein and absolute neutrophil count from baseline; 24h sputum volume and colour; emergence of resistance among baseline pathogens	• Ciprofloxacin for inhalation 32.5mg twice/day • Inhalation of matching placebo twice/day	Well tolerated and significantly reduced total sputum bacterial load at the end of treatment compared with placebo (-3.6 Log

			and emergence of new potential respiratory pathogens		unit reduction)
Ciprofloxacin DPI (BAYQ3939) [RESPIRE-1] [14]	III	Time to first exacerbation over 48 weeks after baseline	Mean number of exacerbations per patient over 48 weeks after baseline, pathogens present at baseline and eradicated at 48 weeks, change of SGRQ score from baseline, new pathogens at 48 weeks, not present at baseline, changes of FEV_1 from baseline, adverse events: number and extent.	• Ciprofloxacin DPI 32.5mg inhaled twice daily intermittently administered for 28 days on/ 28 days off for 6 cycles • Placebo inhaled twice daily intermittently administered for 28 days on/28 days off for 6 cycles • Ciprofloxacin DPI 32.5mg inhaled twice daily for 14 days on/ 14 days off for 14 cycles • Placebo inhaled twice daily intermittently administered for 14days on/14days off for 14 cycles	Full data not published yet
Ciprofloxacin DPI (BAYQ3939) [RESPIRE-2]	III	Time to fist exacerbation over 48 weeks after baseline	Mean number of exacerbations per patient over 48 weeks after baseline, pathogens present at baseline and eradicated at 48 weeks, change of SGRQ score from baseline, new pathogens at 48 weeks, not present at baseline, changes of FEV_1 from baseline, adverse events: number and extent.	• Ciprofloxacin DPI 32.5mg inhaled twice daily intermittently administered for 28 days on/ 28 days off for 6 cycles • Placebo inhaled twice daily intermittently administered for 28 days on/28 days off for 6 cycles • Ciprofloxacin DPI 32.5mg inhaled twice daily for 14 days on/ 14 days off for 14 cycles • Placebo inhaled twice daily intermittently administered for 14days on/14days off for 14 cycles	Full data not published yet
Ciprofloxacin for inhalation [ORBIT-1] [19]	II	Mean change in PA density in sputum log10	Microbiological efficacy; time to/number of/severity of/time to resolve exacerbations; changes in spirometry;	• Ciprofloxacin for inhalation 3ml/day by inhalation for 28 days • Ciprofloxacin for inhalation 2ml/day	2ml and 3ml doses found to be equipotent,

		CFU/g of sputum from baseline to day 28	QoL; safety and tolerability.	by inhalation for 28 days • Placebo 3ml/day by inhalation for 28 days • Placebo 2ml/day by inhalation for 28 days	significantly reducing PA density in sputum with excellent safety and tolerability
Dual Release Ciprofloxacin for inhalation (DRCFI) [ORBIT-2] [18]	II	Mean change in PA density in sputum log10 CFU/g of sputum from baseline to day 28 [timeframe: 168 days]	Microbiological efficacy; time to/number of/severity of/time to resolve exacerbations; changes in spirometry; QoL; safety and tolerability.	• DRCFI once daily made up of liposomal ciprofloxacin for inhalation 150mg in 3ml and free ciprofloxacin 60mg in 3 ml provided in separate vials for 3 cycles of 28 days on / 28 days off • Placebo once daily made up of control liposomes 15mg in 3ml and normal saline 0.9% in 3ml for 3 cycles of 28 days on / 28 days off	Well tolerated and significantly reduced total sputum bacterial load at the end (-4.2 log unit reduction) of treatment compared with placebo
Dual Release Ciprofloxacin for inhalation (DRCFI) [ORBIT-3]	III	Time to first exacerbation [timeframe: 1 year]	Number of exacerbations [timeframe: 1 year]	• DRCFI once daily for 28 days on / 28 days off for six cycles • Placebo Liposomes for inhalation (PLI) once daily for 28 days on and 28 days off for six cycles	Full data not published yet
Pulmaquin (liquid mixture of liposomally encapsulated and un-encapsulated ciprofloxacin) **[ORBIT-4]**	III	Time to first pulmonary exacerbation from baseline [timeframe: 48 weeks]	Number of exacerbations [timeframe: 1 year]	• Pulmaquin once daily 28 days on/28days off for 6 cycles • Placebo (liquid formulation of empty liposomes) once daily for 28 days on/28 days off for 6 cycles	Full data not published yet

4.0 Safety and Tolerability

The main side effects are related to oral Ciprofloxacin. The most common side effects experienced are nausea, vomiting and diarrhoea. Headache, dizziness, insomnia, skin rash or abnormal liver function can be more serious complications related to its use. The most concerning side-effects are peripheral neuropathy and tendonitis which can cause permanent damage [11]. There is limited data available on side effects with inhaled ciprofloxacin but expected side effects will be local side effects including oro-pharyngeal thrush and bronchospasm but its tolerability and side effect profile will come clearer following publication of the phase 3 trials.

5.0 Regulatory affairs

There are currently no licensed inhaled or nebulised drugs for use in bronchiectasis. The ongoing trials if they meet their primary endpoint will then likely meet regulatory approval for licensing for use in bronchiectasis.

6.0 COMMENTARY – EXPERT OPINION

1. **What, if any, improvement does the drug hold over other therapies?**
   The use of specially formulated preparations of ciprofloxacin is designed to achieve optimal deposition in the lungs. Long term treatment is aimed to reduce chronic bacterial suppression, although eradication would be the preferred outcome. Inhaled or nebulised ciprofloxacin over 28 days achieved an excellent log unit reduction in microbial load (-3.4 to -4.1 log units at 28 days), similar to aminoglycosides (-4.6 log units) and both superior to the microbial load reduction with amikacin (-0.4 log units), colomycin (-1.4 log units) and aztreonam (-2.6 log units) [6]. Long term studies are however needed to assess whether this impacts on reducing exacerbations, the endpoint required by EME and FDA for licensing purposes.

2. **What, if any, impact is the drug likely to have on current treatment strategies?**
   To date, colostin [25] and gentamicin [5] has the most robust data for proven effectiveness in bronchiectasis. Nebulised gentamicin is inexpensive and reduced exacerbations in a single centred study, but treatment must be continuous for its ongoing efficacy. The Colsitin trial showed it prolonged the time to first exacerbation but only in patients that complied with treatment $\geq$80% [25]. Cost-effectiveness studies are important and necessary in guiding long term therapy. In addition, there have been no head to head trials comparing inhaled or nebulised antibiotics with long term macrolide therapy so there is no data available to determine which long term treatment strategy (anti-inflammatory or antibiotic) should be instituted first.

   Currently inhaled or nebulised ciprofloxacin has no impact on current treatment strategies. The two main therapeutic long term antibiotics that clinicians currently use are long term macrolide therapy or long term nebulised antibiotics. The long term nebulised antibiotics used internationally, despite being unlicensed, are nebulised gentamicin, colomycin and tobramycin.

3. **How likely are physicians to prescribe the drug?**
   Currently physicians are unlikely to prescribe inhaled/nebulised ciprofloxacin as there are suitable alternatives as highlighted above. The issue of developing resistance, which may make oral ciprofloxacin ineffective for future exacerbations, is an important consideration, especially in the more severe *P. aeruginosa* colonised cohort of patients and so the outcome of the phase III studies are eagerly awaited.
   The situation will however change if there is regulatory approval for inhaled or nebulised ciprofloxacin.

4. **What data is still needed?**
   Results from the Phase III ongoing trials will be key for the outcome of the drugs but additionally, cost-effectiveness and longer-term studies will be necessary to determine the viability of the drug. Head to head studies are needed to decide on the optimum inhaled or nebulised antibiotic and their place with or without long term macrolide therapy. It is also important to determine what treatment is viable for acute exacerbations due to *Ps. aeruginosa* as a fear is that using inhaled or nebulised ciprofloxacin will prevent the use and efficacy of oral ciprofloxacin for exacerbations due to *P. aeruginosa*.

5. **Where is the drug likely to be in 5 years' time?**
   There are many unanswered questions about long term inhaled or nebulised antibiotics. The correct target group needs explored, but in our opinion most will focus treatment in patients with chronic infection with *P. aeruginosa* or non-fermenting gram negative organisms. There will be other potential agents that may also be licensed such as inhaled or nebulised gentamicin, colomycin, tobramycin and perhaps levofloxacin and amikacin. As the nebulised aztreonam study failed to reach its primary endpoint [24], it is unlikely that this will be available for routine use in bronchiectasis. The outcome of the current phase III trials mentioned above will be instrumental in deciding the place of inhaled/nebulised ciprofloxacin amongst the other currently used inhaled antibiotic therapies.
   There may be strategies that look at the optimum duration of inhaled antibiotic therapy and whether treatment will be on/off for 14 or 28 days or continuous treatment. Our belief is that continuous treatment is likely to be the optimum regimen to maintain bacterial suppression and therefore have greater impact on reducing exacerbations. Whether continuous regimens have an adverse impact on subsequent resistance patterns compared with on-off regimens remains to be seen. The long term impact if resistance does develop is not known.
   It will also be interesting to see if there is any added benefit with co-administration of oral macrolide therapy.
   In our opinion, for those chronically infected with *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, long term oral antibiotics should be the first line long term treatment. Evidence to date would be that such patients with recurrent infections having at least three exacerbations per

year will be better to have first line treatment with a long-term macrolide as there is a paucity of randomised controlled trials evaluating other long term oral targeted antibiotics. An alternative, in our opinion is that such patients may also benefit from a targeted long term oral antibiotic such as amoxicillin (in beta lactamase negative patients) or amoxicillin with clavulanic acid or doxycycline (for beta lactamase producing organisms) but randomised controlled trials are needed.

The next 5 years will likely in our opinion start to provide licenced therapies for patients with bronchiectasis.

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* Inhaled Colistin reached the primary outcome in patients that complied with therapy.

Validation of the incremental shuttle walk test as a clinical endpoint in bronchiectasis

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Take home message: Incremental Shuttle Walk Test is an objective clinical endpoint in bronchiectasis

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Abbreviations

Incremental Shuttle Walk test (ISWT)
St George's Respiratory Questionnaire (SGRQ)
Leicester Cough Questionnaire (LCQ)
Quality of life questionnaire-bronchiectasis (QOL-B)
6minute walk test (6MWT)
Bronchiectasis Severity Index (BSI)
Chronic obstructive pulmonary disease (COPD)
Forced expiratory volume in 1 second (FEV_1)
Forced vital capacity (FVC)
Pseudomonas. Aeruginosa (PA)
Potentially pathogenic microorganisms (PPM)
Mixed normal flora (MNF)
White cell count (WCC)
Estimated sedimentation rate (ESR)
C-reactive protein (CRP)

Abstract
Rationale: There is a need for a validated clinical endpoint to assess response to therapies in bronchiectasis.
Objective: To assess the reliability, validity and responsiveness of the incremental shuttle walk test (ISWT) as a clinical endpoint in bronchiectasis.
Methods and measurements: Reliability: In clinically stable patients (n=30) the ISWT was performed twice; 6months apart. Validity: Correlation between the St George's Respiratory Questionnaire (SGRQ) and ISWT (n=94) was performed. The 1-year gentamicin study was reanalyzed to assess the area under the curve (percent change of ISWT with a 4 or more-unit improvement in total SGRQ). Responsiveness: ISWT was performed pre-and post-14 days antibiotics for an exacerbation (94 oral and 30 intravenous courses, n=124) and reanalysis of the 1-year Gentamicin study,n=57.
Main Results: The ISWT did not significantly change over 6-months whilst clinically stable.
The ISWT correlated inversely with the SGRQ ($rs=-0.60$, $p<0.0001$), Bronchiectasis Severity Index score ($rs=-0.44$, $p<0.0001$) and sedentary time ($rs= -0.48$, $p=0.0007$) but correlated with physical activity ($rs=0.42$, $p=0.004$). The area under the curve for % change in ISWT with 4 or more-unit improvement in SGRQ was 0.79 (95% CI 0.66-0.91), $p=0.001$. A threshold of 5% improvement ISWT has a 92% sensitivity but 50% specificity, and from the responsiveness studies would capture 73% of all patients.
Conclusions: This study has confirmed the ISWT to be reliable, valid and responsive to change in patients with bronchiectasis. The authors propose at minimum clinically important difference of 5% improvement in ISWT would be a useful objective endpoint to assess therapies in bronchiectasis.

Introduction
There is an urgent need for objective endpoints to assess progression and response to treatment in bronchiectasis. To date, 24-hour sputum volume, microbial clearance,

C-reactive protein, quality of life assessed by the St George's Respiratory Questionnaire (SGRQ) [1], the Leicester Cough Questionnaire (LCQ) [2], the Bronchiectasis health questionnaire [3] & the Quality of life questionnaire-bronchiectasis (QOL-B) [4, 5] have been found to be useful clinical endpoints [6]. Qualitative bacteriology, and not quantitative bacteriology which is only used in phase 2 studies, [7] is often used in routine clinical practice and can be difficult to interpret in chronically colonised patients [8]. 'Exacerbation frequency' or 'mean exacerbations' are being used only as endpoints in randomised clinical trials, [9-12] with recent consensus on the definition of an exacerbation for use in clinical research being published [13]. The forced expired volume in 1 change (FEV_1) change has shown inconsistent results between studies and not thus a useful endpoint [6, 9-12]. There are limitations with each of these endpoints.

An endpoint assessing functional capacity may be a useful endpoint which is clinically meaningful. The ISWT and 6-minute walk test (6MWT) are used to assess functional capacity. The authors chose the objective assessment of functional capacity, ISWT as opposed to the self-pacing of the 6MWT. Both may be useful, but a study in cystic fibrosis comparing the two tests found patients covered more distance with the ISWT and heart rate and dyspnoea scores were significantly higher in the recovery phase of the ISWT than with the 6MWT ($p<0.05$) [14]. In a small study (n=37) in patients with bronchiectasis the minimum clinically important difference was thought to be 35m using the anchor-based method (AUC 0.88, 95% CI 0.73-0.99) and 37m using the distribution-based method [15].
The aim of this paper is to assess the use of ISWT as a reliable indicator of disease severity, its response to treatment in bronchiectasis as an objective clinical endpoint and its minimum clinically important difference (MCID).

Methodology

Ethics
Ethical approval was granted by the West of Scotland Research ethics service, REC reference 13/WS/0230. All patients provided informed written consent and were recruited from a single centre. This approval applies to all the studies listed except reanalysis of the Gentamicin study by Murray *et al* [16] which had separate ethical approval. – Clinical Trials Registration number NCT00749866.

Study design
The ISWT was validated by assessing its reliability, validity and responsiveness. Each of these parameters was assessed separately by using several sub-studies (Figure 1). Every patient in each of the studies had previously performed the ISWT as part of routine clinical practice and was familiar with how the test was conducted. A practice walk was therefore not necessary. During each sub-study, no changes to medications or overall management of the patients (new airway clearance techniques or enrolment into pulmonary rehabilitation) was made other than the antibiotic described in the sub-study used to treat exacerbations.

1). The reliability of the ISWT was assessed by inviting 30 patients from routine outpatient bronchiectasis clinics (varying severity) to complete the incremental

shuttle walk test 6 months apart when they were clinically stable, defined as no antibiotic use in the last month. There was no change made to their medical management of bronchiectasis during this time.

2). The validity of the ISWT was assessed by correlating the distance walked by patients with SGRQ score. This has been validated for use in bronchiectasis and measures quality of life. 94 different patients with bronchiectasis were recruited prospectively over an 18month period. They were seen at three different time points to perform both the ISWT and complete the SGRQ: (VO) when clinically stable, (VS) start of an exacerbation and (VE) end of exacerbation after treatment with 14 days of oral antibiotics (VE). ISWT results were also correlated with the Bronchiectasis Severity Index (BSI) scores [17] of these patients to assess disease severity. In a sub-study, 49 of these patients (mixture of stable and start of exacerbation) were asked to wear an activity monitor on their arm during waking hours for a period of 14 days. The physical activity time and sedentary time recorded was correlated with the ISWT.

To assess the utility of the ISWT, the authors used the anchor method using a 4 or more-unit improvement in total SGRQ as a clinically significant important improvement in quality of life [1]. The 1-year gentamicin study [16] was reanalyzed to assess the area under the curve (percent change of ISWT with a 4 or more-unit improvement in total SGRQ). This study was selected as there was significant improvement of 1-year nebulised gentamicin compared with the group that received nebulised saline.

3). The responsiveness was assessed by analysing the change in ISWT in patients on short term antibiotic therapy for exacerbations. Short-term antibiotic therapy is the mainstay for treating exacerbations and known to be effective. 94 patients (recruited as part of (2)) received 14days oral antibiotics and performed the ISWT at the start and end of exacerbation. An additional 30 patients receiving 14days of intravenous antibiotic therapy for an exacerbation (as per the British Thoracic Society guidelines [18]) were recruited and performed the ISWT pre and post therapy. Patients on oral or intravenous short-term therapy also had their spirometry checked, serum inflammatory markers, sputum colour [19] and volume recorded at the time of the visits. Responsiveness was also assessed in patients on long-term therapy, by retrospectively re-analysing a study where a different cohort of patients were given either nebulised 0.9% saline or nebulised gentamicin 80mg twice daily for 12months, n=65 [16]. Again, the ISWT was performed pre- and post therapy.

Incremental shuttle walk test

The test was performed per standard test procedure [20] and consisted of a 10-metre shuttle course on a flat surface with the walking speed controlled using pre-recorded audio signals. At the start and end of the ISWT all patients had their heart rate, oxygen saturations and dyspnoea score according to the modified BORG dyspnoea scale [21] recorded in accordance with standardised guidelines (2002) [22]. The successful completion of distance walked in our study was recorded in increments of 10m. Difference in ISWT walked between visits are presented in groups of ‘deterioration/no change’, ‘1-29m improvement’, ‘30-59m improvement’, ‘60-89m

improvement' and '≥90m improvement'. As baseline distance varied amongst patients, the percentage change in ISWT distance was also calculated and presented in groups of 'deterioration', 'no change', '0-4.99% improvement', '5-9.9% improvement' and '≥10% improvement' change.

Activity monitors
Patients were asked to wear the SenseWear Armband (BodyMedia Inc., Pittsburgh, USA) on the right triceps area. The patients were asked to keep it on for 8 days minimum and to only remove it during showering or bathing. The handedness of the patient was recorded.

Lung function
We measured pre-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and ratio FEV_1:FVC by spirometry according to standardized guidelines [23].

Sputum
Sputum colour was recorded according to a visual colour chart rating. Mucoid sputum was rated 1, mucopurulent rated as 2 and purulent sputum rated as 3 or 4 depending on colour [19]. 24hour sputum volume was recorded by asking patients to expectorate solely into a universal container for 24hours. Qualitative and quantitative microbiology was performed on all samples pre- and post- 14 days of antibiotics.

The minimum clinically important difference
The anchor method was used to determine the minimum clinically important difference using a 4 or more unit rise in SGRQ as a clinically significant improvement of quality of life.

Statistical analysis
All data were analysed using Graphpad prism version 5.0a (Graphpad software, San Diego, CA, USA). For demographic and clinical variables, data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables unless specified otherwise. A Bland-Altman test was used to show repeatability over time with bias and 95% limits of agreement calculated and intraclass correlation coefficient calculated (study 1). Correlations were assessed using the Spearman correlation. The area under the curve was calculated using SPSS® version 24. A P value <0.05 was considered statistically significant for each analysis. Comparison of changes within a group i.e before and after treatment was calculated using Wilcoxon signed rank test and we compared categorical data between groups with the $\chi 2$ test. An unpaired *t* test was used to calculate the change from baseline to 12months in ISWT between patients assigned gentamicin and nebulised saline with results displayed as mean difference (95% confidence interval). The MCID was calculated using the anchor method.

Results

Reliability

The aetiologies of bronchiectasis in the 30 patients recruited were 70% idiopathic, 13% post infection, 13% autoimmune disease and 3% IgG2 subclass deficiency. Patient baseline characteristics are outlined in table 1.

Table 1. Baseline characteristics

Characteristics	Reliability (n=30)	Responsiveness IV (n=30)	Responsiveness oral (n=94)
Age (years)	59.1 (11.4)	62.4 (11.4)	65.6 (10.7)
Female	47%	47%	54%
Never smoked **Ex – smoker** **Current**	60% 40% 0%	70% 30% 0%	62% 36% 2%
FEV_1 (L) **Predicted FEV_1 (%)**	2.2L (0.9) 73.9% (24.5)	1.58 (0.84) 56.6% (24.9)	1.90 (0.75) 75.8% (23.9)
FVC (L) **Predicted FVC (%)**	3.2L (1.1) 86.4% (25.6)	2.53 (0.9) 72.7% (23.2)	2.85 (0.92) 91.4% (22.3)
Sputum microbiology	Pseudomonas 0% Other PPM 40% Mixed normal flora 57% No sputum 3%	Pseudomonas 27% Other PPM 60% Mixed normal flora 17%	Pseudomonas 13% Other PPM 76% Mixed normal flora 20%
Inhaled corticosteroids **Long term oral (>28 days) oral corticosteroids**	60% 7%	80% 10%	70% 2%
COPD	0%	7%	14%
Co-existing asthma	57%	43%	49%
Long term (>28 days) antibiotics	3%	10%	7%

Table 1. Baseline characteristics of patients in this study. Results are presented as mean (SD) or %. IV: intravenous, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, PPM: potentially pathogenic microorganisms, COPD: chronic obstructive pulmonary disease.

At the first visit, patients walked a median (IQR) distance of 390m (225m - 462.5m). 6 months' later patients were recalled and the test was performed again in an identical manner. Patients walked a median distance of 400m (260m - 480m) p=0.48. The median change in distance walked was 0m (-25m – 50m) with median 0% change (-4.9% - 18.2%). A Bland-Altman plot showed a bias of 1.38m and 95% limits of agreements from -188m to 185m (see figure 2). The intraclass correlation coefficient was calculated to be 0.85 (95% CI 0.68-0.93, p<0.001).

Validity

We correlated the meters walked in the ISWT in all three visits for the 94 patients (282 tests) with the SGRQ (Total and Activity). There was a negative correlation with total and activity scores of the SGRQ with ISWT as shown in figure 3 below; rs=-0.60 (P<0.0001) and rs=-0.64 (P<0.0001) respectively. In the sub-study of 49 patients, the ISWT negatively correlated with sedentary time (rs=-0.48 (P=0.0007)) and positively correlated with physical activity duration (rs=0.42 (P=0.004)) time measured on the activity monitors – see figure 4. The severity of bronchiectasis assessed by calculating the bronchiectasis severity index at baseline also negatively correlated with the ISWT, rs=-0.44 (P<0.0001).

In the 12-month nebulised antibiotic study, the area under the curve for % change is ISWT with 4 or more-unit improvement in SGRQ was 0.79 (95% CI 0.66-0.91), p=0.001. The sensitivity and specificity for the threshold of % improvement ISWT are: for 2% improvement in ISWT, 92% sensitivity and 42% specificity; for 5% improvement in ISWT, 92% sensitivity and 50% specificity; for 7.5% improvement in ISWT, 88% sensitivity and 58% specificity; for 10% improvement in ISWT, 83% sensitivity and 62% specificity.

The authors determined that a clinically useful test would be when we could achieve at least 50% of patients having a 50% improvement in SGRQ or more – see table 2. Using data from exacerbations, a 5% improvement or greater in ISWT would represent the MCID. Although all patients receiving antibiotic therapy improved, Erythrocyte Sedimentation Rate only improved in the group that had a 5% or more improvement in the ISWT (p<0.001). Both groups had improved C reactive protein, but the group that had 5% or more improvement had a greater reduction in C reactive protein (p=0.007, data not shown).

Table 2.

% improvement in ISWT group	**% of 110 patients**	**% with SGRQ improvement (≥4units) within the group**	**AVERAGE SGRQ CHANGE (Units)**
Deterioration	10.9%	25%	-2.08
0.1-1%	6.4%	42.8%	-1.67
1.1-2.4%	1.8%	50%	-5.5
2.5% - 4.9%	6.4%	42.8%	5.29
5-9.9%	9%	60%	-8.28
10-19.9%	25.5%	53.6%	-6.86
≥ 20%	40%	68.1%	-9.86

Table 2: Percentage of patients with different percent improvements in ISWT, after treatment with oral and intravenous antibiotics for exacerbations (data not available in 14), with their average change in SGRQ score (a reduction of 4 or more units is a clinically significant difference).

Responsiveness – Intravenous therapy
30 patients were given intravenous antibiotics for exacerbations of bronchiectasis. Their baseline characteristics are presented in table 1.

Patients were assessed at the beginning and end of intravenous therapy to ensure that improvement after intravenous therapy had occurred. Patients had reduced sputum purulence, reduced sputum volume, improved forced vital capacity, improved white cell count and systemic inflammatory markers erythrocyte sedimentation rate, C reactive protein and improved sputum bacterial clearance – see online supplement table 1. At the start of exacerbation, patients were walking a median distance of 265m (137.5-395.0). After 14 days of intravenous therapy, the median distance walked improved to 335m (190.0-450.0), p=0.004 with the median change 32.5m (-12.5m – 90m) and percentage change 11.9% (-3.7 – 38.9%).

Responsiveness - Oral therapy
94 patients attended for exacerbations that were managed on an outpatient basis with oral antibiotics. Oral antibiotics reduced sputum purulence and volume, improved forced expired volume in 1 second and forced vital capacity, reduced circulating white cell count and systemic inflammatory markers erythrocyte sedimentation rate and C reactive protein and improved sputum bacterial clearance (online supplement table 2). Patients walked a median value of 370m (252.5-540) on the incremental shuttle walk test when clinically stable at the baseline visit. At start of exacerbation the median distance walked was 330m (180-510) with median change in distance -40m (-100-0) and median percentage change -11.1% (-33.1% - 0) compared with pre-exacerbation whilst patients were clinically stable (P<0.0001). After oral antibiotic therapy, the median distance walked was 350m (210-530). The median improvement in distance walked was 50m (10m - 85m) with percentage improvement of 16.3% (3.1% - 33.3%) compared with the start of the exacerbation (P<0.0001). The distance walked at baseline did not differ from the distance walked after oral therapy, suggesting patients may have returned to their baseline level of function (p=0.8).

Responsiveness - Nebulised therapy
In a previous study 65 patients were randomly assigned to nebulised gentamicin 80mg therapy twice daily or nebulised 0.9% saline [16]. They performed an incremental shuttle walk test at baseline and repeated it at 12months of therapy. There was improved incremental shuttle walk in the gentamicin treated group compared with the saline arm with a mean difference 90.4m (95% CI 40.76 – 140m, p=0.0006) and a mean % difference 34.7% (95% CI 12.56 – 56.79, p=0.003).

Change in ISWT with antibiotic therapy
The actual difference in distance walked and percentage change was calculated for each of the treatment groups and shown in figures 5 and 6 respectively. Overall there was 20% of patients that had ‘deteriorated/ no change’ in the ISWT, 14% ‘1-29m’ improvement, 16% ‘30-59m’ improvement; 20% ‘60-89m’ improvement and 30% ‘≥90m’ improvement.
Overall 14% of patients tested showed a ‘deterioration’ in %ISWT distance walked,

6% demonstrated 'no change' on the % ISWT distance walked compared with baseline, 8% of patients showed a '1-4.99%' improvement, 10% had '5-9.99%' improvement and 63% had '≥10%' improvement in the ISWT.

A threshold of 30m improvement would capture 66% of all patients (50% that received 14d intravenous antibiotics, 64% that received 14d oral antibiotics and 89% received 12m nebulised gentamicin). A threshold of 60m would capture 50% (43% of those that received 14d intravenous antibiotics, 48% of those that received 14d oral antibiotics and 63% that received 12m nebulised gentamicin). If a threshold of at least 5% improvement in ISWT is used, this would capture 73% overall (60% of those that received 14d intravenous antibiotics, 69% of those that received 14d oral antibiotics and 96% of those that received 12m nebulised gentamicin). A threshold of at least 10% improvement in ISWT would capture 63% overall (50% of those that received 14d intravenous antibiotics, 61% of those that received 14d oral antibiotics and 81% of those that received 12m nebulised gentamicin).

SGRQ and at least 5% improvement in ISWT

SGRQ scores for 110 patients that received antibiotic therapy were analysed (complete data from 14 patients not available). 62.2% of patients that had at least a 5% improvement in their ISWT distance also had a 4 or more unit rise in SGRQ score (p=0.02). 62.5% of patients that had at least a 10% improvement in their ISWT had a 4 or more unit rise in SGRQ score (p=0.02).

13.2% of patients that had at least a 30m improvement in ISWT distance also had a 4 or more unit rise in SGRQ (p=0.15) and 14% of those with a 60m improvement or more had a significant improvement in SGRQ of 4 units or more (p=0.28).

Figure 1.

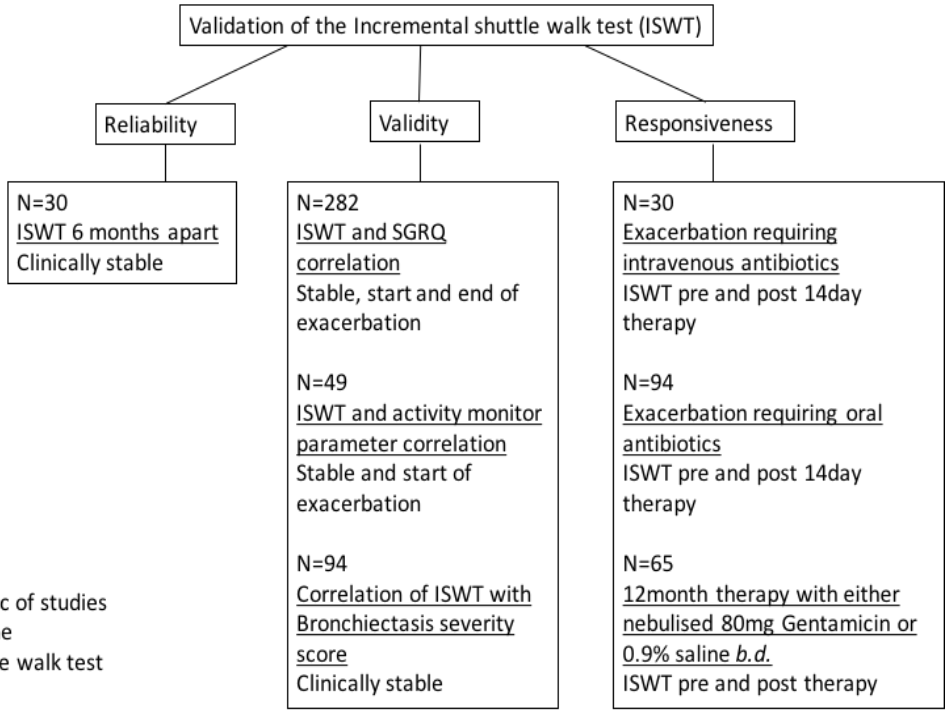

Figure 1. Schematic of studies used to validate the Incremental shuttle walk test (ISWT).

Figure 2.

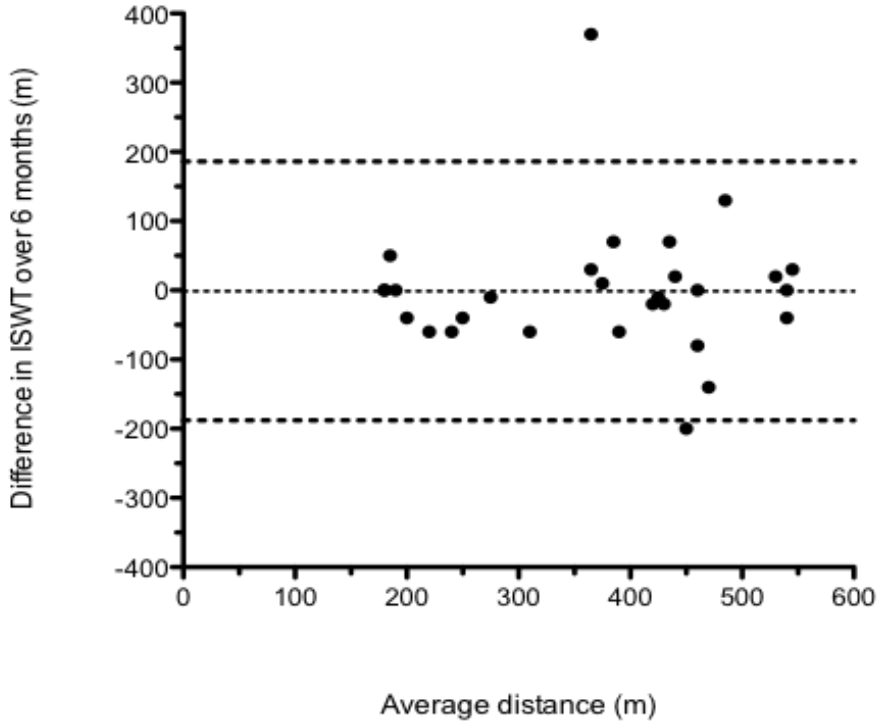

Figure 2. Bland-Altman graph of 30 stable patients that completed the ISWT 6 months apart. The difference versus average distances walked are shown. The middle line represents the mean bias (-1.4m). The upper and lower dashed lines represent the limits of agreement (95% confidence intervals -188.4 – 185.6m).

Figure 3.

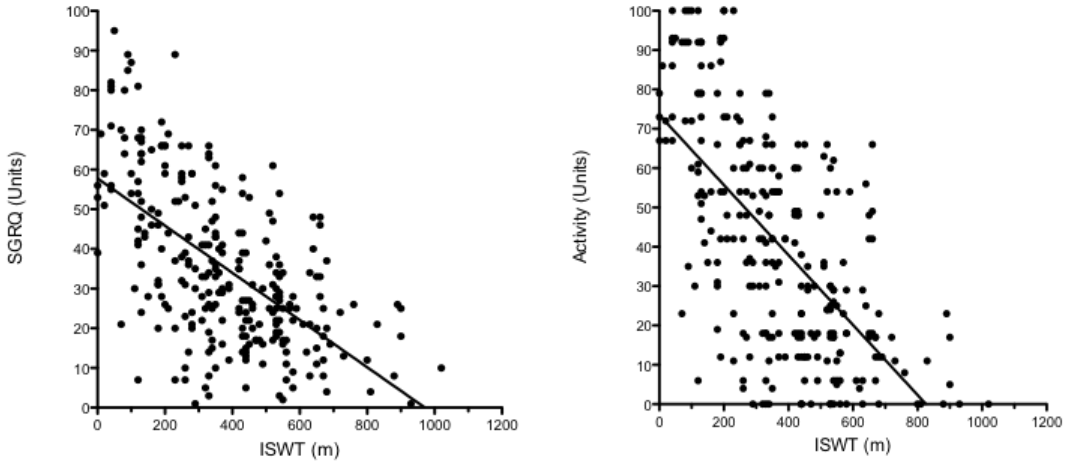

Figure 3. Graphs to show the negative correlations between ISWT distance walked (m) and total SGRQ score (left) and the ISWT distance walked (m) and Activity SGRQ score (right). All results from 94 patients taken at 3 different time points (stable, start and end of exacerbation) are included. rs=-0.60 ($P<0.0001$) and rs=-0.64 ($P<0.0001$) respectively.

Figure 4.

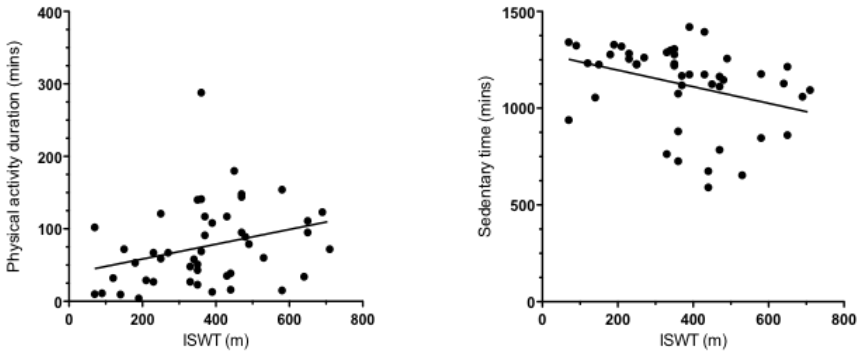

Figure 4. Graphs to show the positive correlation between ISWT distance walked and the amount of time spent doing physical activity (left) and the negative correlation between ISWT distance walked and the amount of time spent being sedentary (right) in a 2-week period, rs=0.42 (P<0.004) and rs=-0.48 (P<0.001) respectively, n=49.

Figure 5.

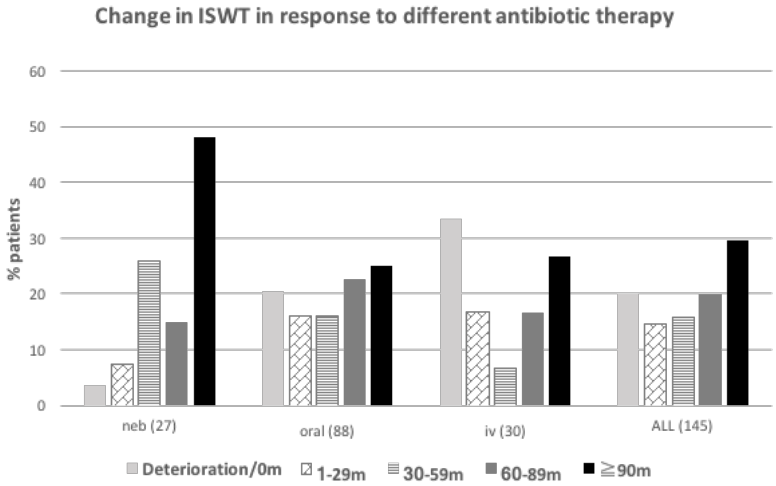

Figure 5. Graph to show the percentage of patients that had different levels of improvement in ISWT walked (m) after either 12months nebulised gentamicin therapy, 14days oral antibiotic therapy, 14days intravenous antibiotic therapy or all patients together.

Figure 6.

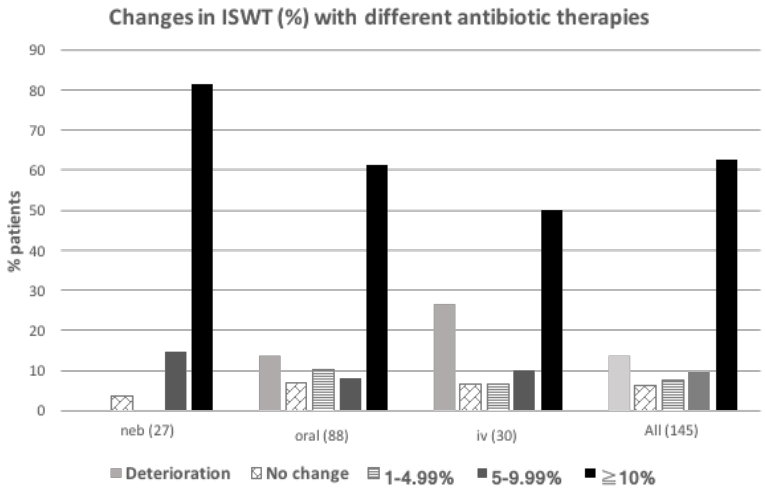

Figure 6. Graph to show the percentage of patients that had different levels of percent improvement in ISWT distance walked after either 12months nebulised gentamicin therapy, 14days oral antibiotic therapy, 14days intravenous antibiotic therapy or all patients together.

Discussion

The main findings of this study support the ISWT to be reliable, valid and additionally, responsive to therapy (both short-term and long-term) in a large cohort of patients with bronchiectasis and therefore supports its use as an objective clinical endpoint that represents functional capacity.

The reliability was assessed over a 6-month period as the proposal would be to use the ISWT to assess both short or long-term therapies. At each time period, patients were stable and had not required antibiotic therapy for at least 28 days. The ISWT was found to be repeatable, in line with previously reported findings [24].

The validity of the ISWT was supported with good negative correlations with both the total and activity SGRQ scores. The SGRQ was chosen as it is already validated for use in bronchiectasis and specifically measures activity as one of its domains. The correlation would never be perfect as the ISWT is an objective measure of exercise capacity whilst the SGRQ is a self-filled questionnaire. In a sub group, there was a moderate correlation with sedentary and physically active duration time measured by activity monitors and further supports the proposal that the ISWT correlates well with other markers of exercise capacity. Overall there has been demonstration of at least moderate validity with the ISWT.

The authors assessed the response to therapies known to work in bronchiectasis and included patients with an exacerbation treated with oral antibiotics and a more severe group receiving intravenous antibiotic therapy. Objective assessments showed patients did improve with short term antibiotic therapy and similarly there were significant improvements in ISWT. The third group chosen was the previously published study assessing the effectiveness of nebulised gentamicin over 1 year to assess the ISWT with effective long-term therapy. Patients felt better with nebulised gentamicin therapy and there was a reduction in overall exacerbations compared

with the group that received nebulised 0.9% saline therapy. There was similarly a significant improvement in the ISWT (in the gentamicin arm) over 1 year [16]. The change in ISWT distance was found to be sensitive to treatment with the above therapies and the authors would therefore recommend that the ISWT be used to assess changes in functional capacity in a longitudinal manner as opposed to a one-off measurement which would be biased by a patient's baseline functional status.

As patients will all have various functional capacities, so in addition to assessing the change in ISWT over time in terms of metre change, the authors also analysed a percentage change. The authors assessed all the responsiveness studies to see whether there was a threshold of improvement in ISWT that could be used for future studies. The best improvement was at least a 30m improvement or 5% improvement in ISWT capturing 66% and 73% of patients overall respectively. Only the percentage improvement group had a clinically significant improvement in SGRQ of 4 units or more. The area under the curve showed that the % ISWT was at least moderate value in predicting a 4 or more-unit improvement in SGRQ. As the cut-off rose, the sensitivity fell and specificity improved. For the MCID, the authors proposed that at least 50% should have a 4 or more-unit improvement in SGRQ and using a threshold of 5% ISWT improvement this was achieved when treating exacerbations of bronchiectasis. The authors would therefore recommend a MCID of 5% change in ISWT to be used as an objective endpoint for assessing response to new and existing therapies.

Limitations of this single-centre study was that only patients that could perform the ISWT were included in this study and although there was a wide spectrum of severity in the included studies, the applicability to very severe bronchiectasis merits further study. For this entire study, only 2.3% were unable to perform the ISWT out of the combined cohort. Another limitation is choosing an anchor that defines a good clinical response. The authors used a 4 unit or more improvement in SGRQ as a good surrogate marker, but there are limitations as this is a self-filled questionnaire.

Previous research by Carmargo *et al* in patients with bronchiectasis has shown the ISWT to be reliable [24] and the incremental shuttle walk test has previously been used to assess the effect of interventions e.g. chest physiotherapy [25] and pulmonary rehabilitation [26, 27] on patients with bronchiectasis but to date it has not been validated for use as an objective clinical endpoint in bronchiectasis.

In conclusion, this study has confirmed the ISWT to be reliable, valid and responsive to change in patients with bronchiectasis. The authors propose at minimum clinically important difference of 5% improvement in ISWT would be a useful objective endpoint to assess therapies in bronchiectasis. External validation is needed for the proposed 5% improvement in ISWT and should be tested in future multi-centred studies.

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analysis and interpretation, the writing and editing of the manuscript.

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The Bronchiectasis Severity Index Score Correlates with Bronchiectasis Disease Severity Using Markers not Included in the Original Scoring System

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Abstract

Background: The Bronchiectasis Severity Index is a 9 parameter prognostic tool that predicts mortality and hospitalisation. There are other clinical endpoints routinely used to assess patients with bronchiectasis.

Aim: To assess the correlation of other markers of severity in bronchiectasis with the bronchiectasis severity index that are not in the original scoring system.

Design and methods: 207 clinically stable patients were stratified according to the bronchiectasis severity index. They all performed a variety of tests including The Leicester Cough Questionnaire (LCQ), The St George's Respiratory Questionnaire, sputum colour analysis, incremental shuttle walk test (ISWT), provided sputum for quantitative bacteriology and inflammatory marker assays, lung function tests and provided whole blood for serum inflammation analysis.

Results: The Bronchiectasis Severity Index correlated well with cough severity as assessed by the LCQ ($p=0.01$), sputum colour ($p=0.02$), 24hr sputum volume ($p=0.0008$), ISWT ($p<0.0001$), bacterial load ($p=0.03$), sputum neutrophil elastase ($p=0.0025$), sputum myeloperoxidase ($p=0.01$), White cell count ($p=0.0046$), Neutrophil count ($p=0.0015$), Erythrocyte sedimentation rate ($p=0.0065$), C-reactive protein ($p=0.0045$) and all lung function parameters including FVC and FEF_{25-75} ($p<0.0001$).

Conclusions: Clinical parameters of sputum characteristics, cough severity, ISWT, sputum and serum inflammatory markers in regular clinical use that are not

incorporated in the BSI score correlate with BSI severity and further work is required to see if they are additive or independent in predicting hospitalisation and mortality.

Introduction

Bronchiectasis is a chronic respiratory condition where the airways are dilated and distorted. This in turn leads to destruction of the mucociliary escalator and a build-up of stagnant mucus. Chronic infection prompts a neutrophilic inflammatory response in the airways which further propagates the 'vicious circle' of infection and inflammation. There is a broad spectrum of disease severity, which until recently was classed as 'mild', 'moderate' and 'severe' with no real accepted criteria for classification.

There is a real need to risk stratify patients to guide the allocation of finite therapies in bronchiectasis and also for predicting prognosis. The Bronchiectasis Severity Index (BSI) grades patients with bronchiectasis from a minimum score of 0 (very mild) to 26 (very severe).[i] The scores are classified into tertiles where 0-4 is a low score, 5-8 as intermediate and $\geqq 9$ is high score. The BSI incorporates 9 variables; age, body mass index (BMI), FEV_1 % predicted, hospital admission in the previous 2 years, number of exacerbations in the last year, MRC dyspnoea score, Pseudomonas aeruginosa colonisation, colonisation with other microorganisms and radiological severity. The BSI has been externally validated and shown to predict mortality, hospital admission, risk of future exacerbations and quality of life.

The presence of some comorbidities (not incorporated in the BSI score) have been associated with more severe disease and can contribute to hospitalisation. McDonnell and colleagues constructed the Bronchiectasis Aetiology Comorbidity Index (BACI) designed to predict the 5-year mortality of comorbidity diagnoses. The hazard ratio (95% CI) for death conferred by a one point increase in the BACI score was 1.18 (1.14 - 1.23), $p<0.0001$. When the BACI score was used in conjunction with the BSI, the combined model was superior overall in predicting 5-year mortality with AUC (95% CI) 0.83 (0.79 – 0.87).[ii]

Furthermore, the importance of the neutrophilic inflammatory response in bronchiectasis has been recognised with more treatments targeting this part of the vicious cycle.[iii,iv,v] There has been some evidence that bacterial load correlates with inflammatory markers and that bacterial load was directly associated with the risk of subsequent exacerbations and the severity of exacerbations.[vi]

The BSI incorporates 9 clinical parameters in its severity score. Components of the BSI were picked based on clinical parameters that are routinely measured in clinical practice. Many other factors other than these assess disease severity in bronchiectasis. The aim of this study is to evaluate whether BSI correlates with endpoints not used in the original scoring system. These include cough severity assess by the Leicester Cough Questionnaire,[vii] sputum colour,[viii] sputum volume, ISWT, quantitative bacteriology, sputum and serum inflammation and lung function including mid expiratory flows and forced vital capacity.

Methodology

Ethics
Ethical approval was granted by the West of Scotland Research ethics service, REC reference 13/WS/0230. All patients provided informed written consent and were recruited from a single centre.

Recruitment and study design
Patients that were clinically stable (no antibiotic therapy for over 6 weeks for an exacerbation prior to selection) were recruited from a dedicated outpatient bronchiectasis clinic in a tertiary hospital (Royal infirmary of Edinburgh). Patient aged 16years and over with radiologically evident bronchiectasis on High Resolution Computer Tomography (HRCT) scan were invited to join and attend a consultation where the below tests were performed. Bronchiectasis severity score (BSI) was calculated for each individual patient according to previous clinical data recorded in routine clinic appointments. All patients underwent the tests below and results analysed for each of the three severity groups 'BSI 0-4 mild', 'BSI 5-8 moderate' and 'BSI ≥9 severe'.

Incremental shuttle walk test
The test was performed per standard test procedure.[ix]

Lung function
We measured pre-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), mid expiratory flows and ratio FEV_1:FVC by spirometry according to standardized guidelines.[x]
Sputum
Sputum colour was graded according to a visual colour chart rating.[8] 24hour sputum volume was recorded by asking patients to expectorate solely into a universal container for 24hours. Qualitative and quantitative microbiology was performed on all samples.[xi]

Quality of life
All patients were asked to complete The Leicester Cough Questionnaire[7] and the St George's Respiratory Questionnaire.[xii]

Sputum inflammatory markers
Whole sputum samples were centrifuged to produce supernatants on which myeloperoxidase (MPO), neutrophil elastase (NE) and interleukin – 8 (IL-8) were tested. Myeloperoxidase activity was measured with a chromogenic substrate assay and free elastase activity by spectrophotometry with a synthetic substrate (methoxysuccinyl-Ala-Ala-Pro-Val paranitroanilide; Sigma, Gillingham, UK) and interleukin 8 measured using commercially available specific ELISAs (R&D Systems, Oxford, UK).[xiii]

Blood inflammatory markers
Venous blood sampling was taken to monitor full blood count, urea and electrolytes, Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Statistical analysis

All data were analysed using Graphpad prism version 5.0a (Graphpad software, San Diego, CA, USA). For demographic and clinical variables, data are presented as mean (standard deviation) if data was normally distributed and median (interquartile range) if not normally distributed for continuous variables and n (%) for categorical variables unless specified otherwise. A P value <0.05 was considered statistically significant for each analysis. Comparison of changes between groups was performed by Analysis of Variance (ANOVA) statistical test or the Kruskal Wallis test if data was not normally distributed.

Results

207 patients were recruited from a tertiary hospital outpatient clinic. Baseline characteristics are displayed below in table 1. Aetiology of bronchiectasis is shown in table 2.

Quality of life

The Leicester Cough Questionnaire (LCQ) assesses quality of life based on the impact of cough severity across the domains of physical, psychological and social items. The St George's Respiratory Questionnaire (SGRQ) assesses quality of life based on symptoms, activity and psychosocial impact. Both quality of life assessments correlated with BSI severity; SGRQ: p<0.0001 and the LCQ: p=0.01 – see figure 1. The median (IQR) of LCQ results for each BSI tertile were: 19 (16-20), 18 (14-20) and 16 (13-19) respectively. The median (IQR) for the SGRQ for each group was: 26 (17-37), 29 (16-44) and 44 (30-66) respectively.

Figure 1.

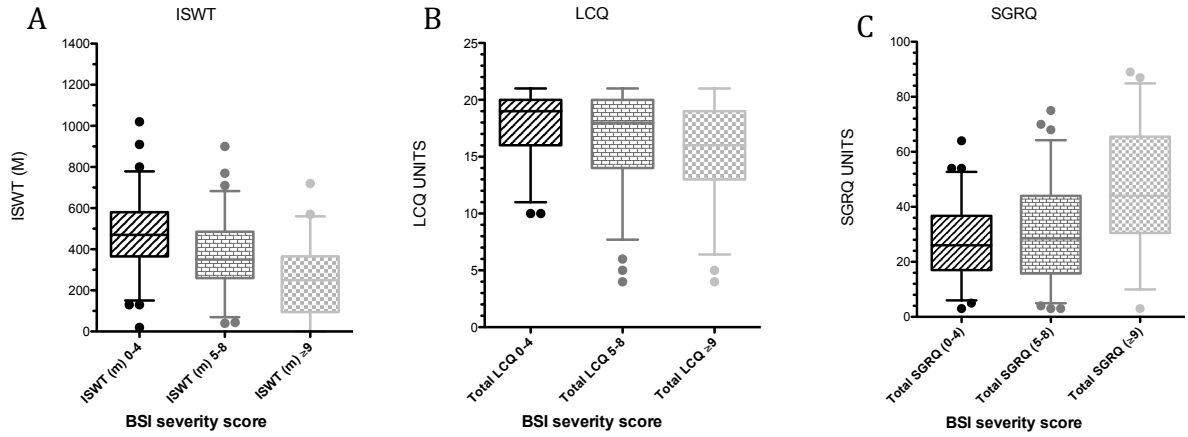

Figure 1. Quality of life in clinically stable patients with bronchiectasis in 'mild, 0-4' 'moderate, 5-8' and 'severe, ≥9' BSI groups shown as boxplot with median (IQR) and 5-95% CI, A: Incremental shuttle walk test (ISWT), p<0.0001, B: Leicester Cough Questionnaire (LCQ), p=0.01, C: St George's Respiratory Questionnaire (SGRQ), p<0.0001. The minimal clinical important difference is 1.3 Units for the Leicester Cough Questionnaire and 4 Units for the St George's Respiratory Questionnaire.

Sputum characteristics
There were associations with increasing 24hr sputum volume and with increasing sputum purulence with higher BSI scores – see Table 3.

Incremental shuttle walk test
The distance walked in the incremental shuttle walk test was significantly lower in the progressive BSI tertiles of severity. In BSI group 0-4 the mean (SD) ISWT distance was 484.2m (183.9), for group 5-8 was 372.4m (180.5) and for those with a score of 9 and over had deteriorated to 250.1m (179), $p<0.0001$ – see Figure 1.

Bacteriology
There was a significant increase in bacterial load with increasing severity of BSI from groups 0-4, 5-8 and ≥9 with median (IQR) counts of 0 CFU/ml (0 - $9.3x10^6$), $0.1x10^5$ CFU/ml (0 – $7.4\ x10^6$) and $1.27x10^7$ CFU/ml ($0.02\ x10^6$ – $1.0\ x10^8$) respectively, $p<0.0001$ – see Table 3.

Sputum inflammatory markers
With increasing BSI score, there were increasing levels of sputum free neutrophil elastase (p=0.0025) and sputum myeloperoxidase (p=0.01). There was no association with Interleukin-8 (p=0.57), see Table 3.

Serum inflammatory markers
There was a significant association between increasing BSI severity group and white cell count (WCC) (p=0.0046), neutrophil count (p=0.0015), Erythrocyte sedimentation rate (p=0.0065) and C-reactive protein (p=0.0045) whilst clinically stable - see Table 3.

Lung function tests
Forced expiratory volume in 1 second (FEV_1), percent predicted of FEV_1 (% FEV_1), forced vital capacity (FVC), percent predicted FVC (%FVC), mid expiratory flows at 25-75% (FEF_{25-75}) and percent predicted of FEF_{25-75} (%FEF_{25-75}) was tested in all patients. All lung function parameters deteriorated with increasing BSI severity group, $p<0.0001$ – see Table 3. Mean (SD) for each BSI group 0-4, 5-8 and ≥9 for FEV_1: 2.42L (0.81), 1.94L (0.63) and 1.61L (0.64); %FEV_1: 87.1% (20.1), 80.2% (24.7) and 64.2% (21.9); FVC: 3.4L (0.95), 3.0L (0.79) and 2.7L (0.97); %FVC: 101.5% (20.6), 99.3% (25.0) and 80.4% (23.8); FEF_{25-75}: 1.7L (1.0), 1.2L (0.7) and 0.9L (0.6); %FEF_{25-75}: 52.8% (29.1), 41.7% (23.6) and 31.1% (20.6) respectively.

Discussion
The bronchiectasis severity index score is internationally validated for predict 30-day mortality rate and hospitalisation. The authors have found that in addition the BSI links with sputum purulence, 24hr sputum volume, quantitative bacterial load, lung function, functional capacity, quality of life, sputum and serum inflammatory markers.

These other endpoints have been shown to be of importance previously but have not to date been linked with the bronchiectasis severity index.

Sputum colour was investigated by Murray and colleagues in stable patients with bronchiectasis. They reported good reliability of sputum colour between patients and clinician and that it was predictive of bacterial colonisation ($p<0.0001$). They also reported independent factors associated with sputum purulence were bacterial colonization, radiological severity with varicose or cystic bronchiectasis, FEV_1 less than 80% predicted and diagnosis of bronchiectasis aged less than 45yrs old.[8]

Bacterial load has been evaluated with conflicting evidence in the literature. Tunney and colleagues reported that bacterial load did not change with exacerbations although the sample size was small (n=14) and they did not measure bacterial load during clinical stability and during an exacerbation in the same patients.[xiv] Chalmers and colleagues reported there was a direct relationship between bacterial load and the severity of exacerbations and risk of subsequent exacerbations. They linked bacterial load with airways inflammation and systemic inflammation when assessing both inpatients and outpatient exacerbations. Furthermore, they reported the reduction of bacterial load with short and long term antibiotic therapy.[6]

Lung function in bronchiectasis has not been well characterised in bronchiectasis. A study by Guan and colleagues have reported in 142 stable patients that the variables associated with a 50% or lower FEV_1 were radiological severity ($p<0.01$), presence of *Pseudomonas aeruginosa* ($p<0.01$) and the presence of symptoms for 10 or more years ($p=0.01$). They also noted differences in FEV_1 and FVC between exacerbations and convalescence in a small subgroup ($p<0.05$).[xv] Martinez-Garcia and colleagues also investigated FEV_1 decline in 76 stable patients. They also found accelerated decline in lung function to be associated with more severe exacerbations, the presence of *Pseudomonas aeruginosa* in sputum and systemic inflammation.[xvi]

The ISWT is an objective measurement of functional capacity and has been previously shown to remain stable in patients with clinical stability i.e. no change to medications and no exacerbation 6 months apart.[13] It has also been shown to improve with long-term nebulised antibiotics[11] and anti-inflammatory treatment.[13] The authors demonstrated that the ISWT mean distances deteriorated significantly with increasing severity of BSI. A recent study has validated the ISWT as a clinical endpoint in bronchiectasis and found a 5% difference in distance walked to be the minimum clinically important difference (MCID). They found the ISWT to correlate with severity, deteriorate with exacerbations and improve with treatment.[xvii]

The Leicester Cough Questionnaire (LCQ) (range 3-21, 3 most severe cough severity) and the St George's Respiratory Questionnaire (SGRQ) (range 0-100, 100 most severe) have both been validated as clinical endpoints for use in bronchiectasis.[7, 12] The MCID for the LCQ is 1.3units and for the SGRQ is 4units.

The authors believe that severity of bronchiectasis can be due to the degree of sputum inflammation present in the airways which drives the destructive neutrophilic inflammatory response in the vicious cycle of bronchiectasis. Previous studies have shown the importance of airway inflammation and its correlation with higher bacterial loads and subsequent increased risk of exacerbation and with severe exacerbations.[6] Airway inflammatory markers have also been shown to improve

with the use of long term antibiotic use.[6,11] They also reported on the increased airways inflammation present with patients colonised with *Pseudomonas aeruginosa* and in those with more severe radiological bronchiectasis.[6] Elevation of Neutrophil elastase, a product of activated neutrophils has been investigated by Chalmers and colleagues and was found to be associated with the bronchiectasis severity index score ($r=0.49$, $p<0.0001$) but was not independently associated with mortality rates. The authors found neutrophil elastase to rise in exacerbations and have good discrimination for severe exacerbations and all-cause mortality.[xviii]

Relatively little is known about systemic inflammation in patients with bronchiectasis due to the paucity of studies in this area. A study by Wilson and colleagues in 87 stable bronchiectasis patients investigated the correlation of inflammatory markers with radiological severity, lung function, sputum bacteriology and health related quality of life as assessed by the SGRQ. They reported patients have raised inflammatory markers when clinically stable and that inflammatory markers correlate with disease severity; WCC, neutrophil count, ESR and CRP all correlated with radiological severity, WCC and CRP correlated with total SGRQ scores.[xix]

Significance

There have been no prior studies linking the BSI with sputum purulence despite its importance in recognising severity. This is the first study to show increasing sputum purulence links with increasing severity of BSI. The role of quantitative bacteriology has been debated in the literature but this study supports the notion that increasing bacterial load is associated with increased severity of bronchiectasis. The most commonly research lung function parameter is FEV_1 and variables associated with its decline have been investigated. The BSI already incorporates percent predicted FEV_1 as part of its severity scoring system but as this can be affected by other respiratory conditions that patients with bronchiectasis might have such as asthma and COPD, the authors thought it would be prudent to examine other measures of lung function. This is the first study to find all parameters of actual and predicted FEV_1, FVC and FEF_{25-75} correlated with worsening severity of bronchiectasis as assessed by the BSI. Functional capacity in bronchiectasis has not been well studied in bronchiectasis and has only recently been validated in this respiratory condition. This study has shown that the ISWT links with BSI severity in stable patients. The LCQ and SGRQ are validated clinical endpoints in bronchiectasis. This is the first study to show these measurements of quality of life link with BSI severity scoring. Lastly, the BSI is a very clinical tool and does not incorporate any laboratory endpoints that assess inflammation. This study demonstrates that markers of sputum inflammation –MPO and neutrophil elastase and markers of serum inflammation – WCC, Neutrophil count, ESR and CRP all increase with worsening severity of bronchiectasis as assessed by the BSI.

Limitations

Due to the small number of patients in this study, the authors could not explore the added value of these endpoints to BSI to predict 30-day mortality and hospitalisation.

Conclusion

The bronchiectasis severity index stratifies patients into tertiles and has been shown to predict 30day mortality and hospitalization. The authors have shown the BSI scoring of severity to also link with other endpoints of increasing sputum purulence, 24hr sputum volume, bacterial load, functional capacity as assessed by the ISWT, quality of life, lung function parameters of FVC and mid expiratory flows in addition to FEV_1, sputum and serum inflammatory markers. Further work is needed to assess whether these clinical endpoints could be additive or independent to the BSI in predicted mortality and hospitalisation.

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Table 1.

Characteristic	Total patients
Number	207
Mean (SD) Age (yrs)	65.79 (10.6)
Female n (%)	110 (53)
Current smokers **Ex-smokers** **Never**	9 (4) 83 (40) 115 (56)
Mean (SD)	29.09 (35.54)
COPD **Asthma** **Interstitial lung disease** **Lung cancer** **Pulmonary Hypertension** **Inactive ABPA**	35 (17) 23 (11) 3 (1) 2 (1) 1 (1) 21 (10)
IHD	14 (7)
Other cancers	22 (11)
Inhaled steroid **Dose (mean, SD)** **Oral steroid** **Dose (mean, SD)** **Nebulised bronchodilators** **Long-term antibiotics** **Cyclical IV antibiotics** **LTOT**	23 (11) 211mcg (104.4) 12 (6) 7.7mg (5.3) 7 (3) 11 (5) 1 (0.5) 2 (1)

Table 1 shows the baseline characteristics of the 207 stable patients recruited. N (%) or as otherwise stated. BMI: body mass index, COPD: chronic obstructive pulmonary disease, ABPA: allergic bronchopulmonary aspergillosis, IHD: ischaemic heart disease, IV: intravenous and LTOT: long term oxygen therapy.

Table 2.

Aetiology	Number of patients (%)
Idiopathic	92 (44.4)
Post infectious	55 (26.6)
COPD	9 (4.3)
Inflammatory bowel disease	7 (3.4)
Immunoglobulin deficiency	9 (4.3)
Allergic bronchopulmonary aspergillosis	13 (6.3)
Connective tissue disease	7 (3.4)
Aspiration or inhalation or GORD	15 (7.2)

Table to show the N (%) of patients with different aetiologies of bronchiectasis. COPD: chronic obstructive pulmonary disease, GORD: gastric oesophageal reflux disease

Table 3.

Clinical parameter	BSI 0-4	BSI 5-8	BSI≥9	P value
Sputum colour (units)	1.8 (0.8)	2.1 (0.8)	2.3 (0.8)	0.0008
Sputum volume (mls) median (IQR)	2 (0-10)	4 (0-10)	8 (1-15)	0.02
Bacterial load(cfu/ml) median (IQR)	0 (0 - $9.3x10^6$)	$0.1x10^5$ (0 - $7.4x10^6$)	$1.27x10^7$ ($0.02 x10^6$ - $1.0 x10^8$)	0.03
FEV_1 (L)	2.42 (0.81)	1.94 (0.63)	1.61 (0.64)	<0.0001
FEV_1 % predicted	87.1 (20.1)	80.2 (24.7)	64.2 (21.9)	<0.0001
FVC (L)	3.4 (0.95)	3.0 (0.79)	2.7 (0.97)	<0.0001
FVC % predicted	101.5 (20.6)	99.3 (25.0)	80.4 (23.8)	<0.0001
FEF_{25-75} (L)	1.7 (1.0)	1.2 (0.7)	0.9 (0.6)	<0.0001
FEF_{25-75} % predicted	52.8 (29.1)	41.7 (23.6)	31.1 (20.6)	<0.0001
Sputum NE (ng/ml) median (IQR)	2569 (1408-6191)	2600 (1915-3404)	3238 (2013-11552)	0.0025
Sputum MPO(ng/ml) median (IQR)	1719 (859-4718)	1125 (399-3096)	2521 (1159-9544)	0.01
Sputum IL-8 (ng/ml) median (IQR)	14976 (3815-26466)	13257 (6324-30975)	11848 (5935 - 31455)	0.57
WCC $x10^9$/L median (IQR)	6.3 (5.3-7.9)	6.9 (5.5-8.7)	8.0 (6-10.4)	0.005
Neutrophils $x10^9$/L median (IQR)	3.5 (2.6-4.8)	4.0 (3.2-6.2)	5.2 (3.5-7.5)	0.002
ESR (mm/hr) median (IQR)	11 (6-17)	12 (7-18)	17 (8-28.8)	0.0065
CRP (mg/L) median (IQR)	3 (1-7)	3 (1-8.6)	7 (2.5-22.5)	0.0045

Comparison of changes between groups was performed by Analysis of Variance (ANOVA) statistical test or the Kruskal Wallis test if data was not normally distributed. Data presented as mean (standard deviation) unless otherwise specified. CFU/ml: colony forming units/millilitre, NE: neutrophil elastase, MPO: myeloperoxidase, IL-8: interleukin-8, WCC: white cell count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.

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